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No. 1

HYGROSCOPIC EQUILIBRIUM OF RICE AND RICE FRACTIONS¹

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ABSTRACT

An investigation has been made of the rate of sorption and desorption of moisture by rough rice, head rice, bran, polish, and hulls over the range of 11 to 93% relative humidity at 25°C. The hygroscopic equilibrium of these same fractions has been determined over the same relative humidity range. If the relative humidity of the atmosphere at 25°C. is raised from 10 to 90% the moisture content of whole rice and its fractions increases as follows: rough rice from 4.4 to 17.6%, polished rice from 5.2 to 18.8%, bran from 5.0 to 18.0%, polish from 5.3 to 18.0%, and hulls from 3.7 to 15.3%.

In the past 15 years the combine has been increasingly used for harvesting rice. In 1947 more than one-half of the rice crop in Louisiana was harvested by combines (4). This was true of practically all of the other rice-producing areas. Rice when harvested by combine generally has a moisture content of 20% or more and must be artificially dried to 14% moisture to prevent heating and spoilage during storage. This drying operation must be carried out slowly and at relatively low temperatures. Otherwise the rice kernel loses its vitreous character and a large number of kernels will be broken during processing.

Since the moisture content of rice and rice by-products will vary with the atmospheric humidity conditions, and inasmuch as the moisture content seriously affects the storage and processing characteristics of the grain, an investigation was therefore undertaken of the hygroscopic equilibrium of rough rice, head rice, bran, polish, and hulls. Data were obtained on the rates of sorption and desorption of moisture by whole rice and its various component fractions, and the moisture content in equilibrium with air of constant relative humidity over the range of 11 to 93% relative humidity at 25°C. was determined.

¹ Manuscript received June 25, 1948. Report of a study in which certain phases were carried on under the Research and Marketing Act of 1946.

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Materials

The rice used in this investigation was from two separate lots. The first, henceforth to be known as artificially dried rice, was obtained at a rice mill from a mixed lot containing 17.8% moisture which had been dried in a commercial rice drier to a moisture content of 12.8%. This dried rice was processed only to an undermilled product. The second lot, henceforth to be known as naturally dried rice, was of Rexora variety and contained 16.8% moisture as received at the mill where it was immediately processed to a finished polished rice.

Because of the extreme difficulty of quantitatively separating rough rice in the laboratory into head rice, bran, polish, and hulls without bringing about appreciable changes in moisture content, the investigations were conducted on rough rice and the rice fractions obtained from the same lot as it was being processed in a commercial mill. Samples from each milling stream were collected simultaneously, placed in sealed bottles, and taken to the laboratory. Milling data on the entire lot of the artificially dried rice indicated that one barrel (162 pounds) yielded the following products: undermilled head rice, 125.31 pounds (77.35%); bran, 7.80 pounds (4.81%); and hulls and waste, 28.89 pounds (17.83%). In the case of naturally dried rice, the yield from one barrel was as follows: head rice, second heads, and screenings, 105.87 pounds (65.35%); bran, 21.64 pounds (13.35%); polish, 1.35 pounds (0.83%); and hulls and waste, 33.14 pounds (20.46%).

Methods

Saturated solutions of pure salts were used to provide the desired series of relative atmospheric humidities. Spencer (5) lists a number of saturated salt solutions which maintain constant relative humidities over the entire range. Others may be found in the report of the American Paper and Pulp Association (1). The salts used in this investigation and the values of the corresponding relative humidities for their saturated solutions at 25°C. are listed in Table I.

Saturated salt solutions, together with an excess of solid salt, were placed in large desiccators, and samples of the rough rice, head rice, bran, polish, and hulls were suspended above these solutions by means of partitioned wire mats. The desiccators were stored in a room maintained at 25°C. Every few days the desiccators were opened and 5-10 g. duplicate samples of each fraction were removed and weighed immediately in closed moisture dishes. In all moisture determinations the products were heated at 101°C. for 16 hours in a forced-draft oven, reweighed, and the loss in weight calculated as moisture content on the wet basis.

Because the samples were in intermediary stages approaching hygroscopic equilibrium, it was not feasible to apply the vacuum oven method of determining moisture prescribed by the Official Association of Agricultural Chemists (2) which requires grinding the sample to pass a 20-mesh sieve prior to oven-drying.

TABLE I
RELATIVE HUMIDITIES OF ATMOSPHERES IN CONTACT WITH SATURATED
SOLUTIONS OF VARIOUS SALTS AT 25°C.

Salt	Relative humidity
	%
Lithium chloride	11.1
Potassium acetate	22.5
Magnesium chloride hexahydrate	32.5
Potassium carbonate	43.7
Sodium dichromate dihydrate	53.3
Sodium nitrite	64.4
Sodium chloride	75.4
Potassium chromate	86.4
Ammonium dihydrogen phosphate	92.5

This procedure was not applicable, as the samples would undergo changes in moisture content during grinding, thereby making it impossible to obtain a determination of the moisture content at the time the sample was removed from the conditioning chamber. Moisture contents were therefore determined by placing a sample of approximately 5-10 g. of the rice fraction into a previously dried moisture dish, weighing the sample in the closed moisture dish, drying the sample at 101°-103°C. for 16 hours in a forced draft oven, and reweighing. The covers were replaced on the moisture dishes while they were still in the hot oven, and the covered dishes were cooled to room temperature in a vacuum desiccator containing phosphorus pentoxide as the desiccant. All moisture determinations were made in duplicate, and the results were calculated on the wet basis.

The above-mentioned procedure was used in this investigation because the large number of samples involved at each weighing necessitated the use of the forced-draft oven; slight changes in the time interval of drying had very little effect on the final moisture content; and the slope of the moisture-content curve as a function of time was very nearly horizontal at the time interval chosen.

To compare the results obtained by the above-described method with those obtained by the official method (2), an investigation was made of the effect of state of subdivision, time, and the method of drying on the moisture content of the various rice fractions. For this purpose large samples (100-200 g.) of rough rice, polished rice, and hulls were placed in previously tared crystallizing dishes and the

samples accurately weighed. These samples were then allowed to come into equilibrium with atmospheric conditions of 50% relative humidity at 25°C. for a period of one week after which they were reweighed and ground to pass through a 20-mesh sieve. The grinding was made with a laboratory scale mill situated in the same air-conditioned room in which the samples were stored. Duplicate portions of each of the above-described samples in both the ground and unground state were weighed into previously tared moisture dishes and placed in a forced-draft oven kept at 101°–103°C. This oven was so arranged that the samples could be weighed at oven temperature. Weighings were made at intervals up to 150 hours and the moisture content was calculated from the loss in weight. Bran and polish were also equilibrated at 50% relative humidity and 25°C. and dried as described above. In these cases the state of subdivision was already less than 20-mesh; therefore further grinding was unnecessary.

Other samples of each fraction and its ground portion were weighed into moisture dishes and dried in the vacuum oven at 99°C. Since it was not possible to weigh the samples in the vacuum oven, dry air was admitted to the oven, the moisture dish was sealed, placed in a desiccator over phosphorus pentoxide, cooled, and weighed. The samples were then replaced in the vacuum oven and drying was continued at 99°C. for a total of 145 hours.

Results

Comparison of Vacuum Oven and Air-Oven Moisture Methods. The comparative rates of drying of Rexora variety rice and its fractions determined by the two oven methods of drying are given in Tables II and III. The ground material lost weight in the forced-draft oven much faster than the whole rough rice, polished rice, or hulls, and attained a final moisture content approximately 1% higher than that attained by the unground material. The apparent moisture content of the bran continued to rise throughout the drying period. When dried in a vacuum oven, the ground rice fractions lost moisture more rapidly, but the final equilibrium values attained after 145 hours of drying were in better agreement for both the ground and unground fractions than in the forced-draft oven (Table III).

In the vacuum oven method the values for the apparent moisture content of the bran and polish increased to a maximum value in 12.5 hours, after which there was a slight regain in weight and an apparent decrease in moisture content. The same materials dried in the forced-draft oven continued to lose weight throughout the drying period. These differences in behavior may be attributed to the oil content of these materials. Slight oxidation at the double bonds would cause an

increase in weight and an apparent decrease in moisture content while further oxidation would result in decomposition products which could volatilize and the moisture content would appear to increase.

TABLE II
PERCENTAGE OF MOISTURE REMOVED (WET BASIS) FROM REXORA VARIETY
OF RICE AND ITS FRACTIONS DRIED IN A FORCED-DRAFT AIR OVEN
AT 101°-103°C. AT VARIOUS TIME INTERVALS

Drying period, hours	Whole rough rice	Ground rough rice	Whole polished rice	Ground polished rice	Whole hulls	Ground hulls	Bran	Polish
1	7.4	13.9	7.4	12.5	8.2	13.4	10.2	10.4
1	10.0	14.8	10.5	13.4	11.0	14.2	13.4	11.3
1	11.3	15.3	11.7	14.1	12.2	14.5	14.1	11.7
4.5	14.0	16.3	14.2	15.5	13.0	14.8	15.0	12.2
8	14.5	—	14.8	—	—	—	15.2	12.5
11.5	—	16.7	—	16.1	13.2	14.9	—	—
15.5	15.4	—	15.2	—	—	—	15.8	12.7
23.5	—	16.8	—	16.2	13.3	15.0	—	—
28	—	17.0	—	16.4	13.4	15.1	—	—
30.5	15.9	—	15.5	—	—	—	16.4	13.3
48	—	17.1	—	16.4	13.4	15.1	—	—
55	16.2	—	15.6	—	—	—	16.8	13.7
143	16.4	—	15.6	—	—	—	17.6	13.6
168	—	17.3	—	16.5	13.5	15.3	—	—

TABLE III
PERCENTAGE OF MOISTURE REMOVED (WET BASIS) FROM REXORA VARIETY
OF RICE AND ITS FRACTIONS DRIED IN VACUUM OVEN
AT 99°C. AT VARIOUS TIME INTERVALS

Drying period, hours	Whole rough rice	Ground rough rice	Whole polished rice	Ground polished rice	Whole hulls	Ground hulls	Bran	Polish
1	7.0	12.4	7.7	10.5	12.5	13.1	11.9	8.9
2	10.0	14.0	10.9	12.7	13.2	13.6	13.3	10.6
4	12.4	14.9	13.2	14.1	13.4	14.2	14.0	11.4
7	13.7	15.7	14.4	15.2	14.0	14.5	14.5	11.8
12.5	14.6	16.1	15.1	15.7	14.1	14.7	14.5	11.9
21.5	15.2	16.3	15.5	16.1	14.1	14.8	14.5	11.7
39.5	15.8	16.5	16.0	16.6	14.2	14.9	14.3	11.7
103	16.4	16.5	16.5	16.9	14.2	14.8	14.1	11.8
145	16.5	16.4	16.6	16.8	14.2	14.8	14.1	11.8

The results also indicate that the drying procedure used in this investigation yields numerical values for moisture content in rough and polished rice in good agreement with those obtained by drying ground samples of rough and polished rice in the vacuum oven at 99°C. for 7 hours.

Hygroscopicity of Rough Rice and Mill Products. The effect of length of storage at constant relative humidity at 25°C. on the moisture

content of rough rice and its fractions for artificially dried and naturally dried lots of rice is given in Tables IV-VII and in Figs. 1-4, respectively. The rough rice and head rice consist of whole grains, and the rate of diffusion of moisture to the surface is relatively slow. Probably this is why the rough rice and polished head rice do not attain hygroscopic equilibrium as rapidly as do the bran and hulls. This is an im-

TABLE IV
PERCENTAGE OF MOISTURE (WET BASIS) OF ARTIFICIALLY DRIED ROUGH
RICE STORED FOR VARIOUS TIME INTERVALS AT CONSTANT
RELATIVE HUMIDITY

Conditioning period, days	Relative humidity of conditioning atmosphere, %								
	11.1	22.5	32.5	43.7	53.3	64.4	75.4	86.4	92.5
1	11.4	11.4	11.6	11.8	11.8	12.1	12.2	12.3	12.6
2	10.2	10.7	10.9	11.5	11.6	12.0	12.5	12.7	13.2
3	9.4	10.0	10.4	11.1	11.4	12.1	12.6	12.9	13.9
4	8.8	9.6	9.9	10.9	11.4	12.1	12.7	13.2	14.4
5	8.2	9.1	9.8	10.7	11.3	12.0	12.8	13.3	14.9
7	7.1	8.3	9.3	10.4	11.1	12.1	12.9	13.6	15.6
9	6.5	7.9	8.9	10.0	11.0	12.1	12.9	13.9	16.1
11	6.2	7.7	8.8	10.0	11.1	12.2	13.0	14.0	16.3
14	5.7	7.4	8.6	9.9	11.0	12.0	13.0	14.4	17.1
17	5.6	7.4	8.6	9.9	11.1	12.1	13.0	14.7	17.5
21	5.2	7.1	8.4	9.7	10.9	12.0	13.1	15.0	17.7
28	4.9	7.0	8.3	9.6	10.8	12.0	13.1	—	18.0
35	4.9	7.0	8.3	9.8	10.9	12.1	13.1	15.3	18.2
42	4.7	—	—	—	—	—	—	15.4	18.2

TABLE V
PERCENTAGE OF MOISTURE (WET BASIS) OF UNDERMILLED RICE FROM
ARTIFICIALLY DRIED ROUGH RICE STORED FOR VARIOUS TIME
INTERVALS AT CONSTANT RELATIVE HUMIDITY

Conditioning period, days	Relative humidity of conditioning atmosphere, %								
	11.1	22.5	32.5	43.7	53.3	64.4	75.4	86.4	92.5
1	11.9	12.4	12.3	12.7	12.9	13.1	13.3	13.5	13.6
2	10.3	11.5	11.2	12.2	12.7	13.3	13.7	14.0	14.4
3	9.1	10.5	10.6	11.8	12.6	13.3	13.9	14.3	14.8
4	8.1	9.9	10.2	11.5	12.4	13.4	14.1	14.6	15.4
5	7.4	9.2	9.9	11.2	12.4	13.4	14.2	14.7	15.6
7	6.7	8.5	9.4	10.8	12.2	13.4	14.1	15.1	16.3
9	6.2	8.2	9.2	10.7	12.1	13.3	14.2	15.4	16.9
11	6.0	8.1	9.2	10.7	12.2	13.5	14.3	15.5	17.4
14	5.5	7.8	9.0	10.7	12.2	13.4	14.4	15.3	17.9
17	5.3	7.6	8.9	10.7	12.2	13.6	14.5	16.1	18.3
21	5.1	7.5	8.8	10.6	12.2	13.5	14.6	16.4	18.6
28	4.9	7.4	8.9	10.6	—	13.6	14.6	—	19.2
35	4.9	7.4	9.1	10.7	12.2	13.7	14.8	16.8	19.5
42	4.8	—	—	—	—	13.6	14.7	16.8	19.4

TABLE VI
PERCENTAGE OF MOISTURE (WET BASIS) OF BRAN FROM ARTIFICIALLY
DRIED ROUGH RICE STORED FOR VARIOUS TIME INTERVALS
AT CONSTANT RELATIVE HUMIDITY

Conditioning period, days	Relative humidity of conditioning atmosphere, %								
	11.1	22.5	32.5	43.7	53.3	64.4	75.4	86.4	92.5
1	7.5	8.0	8.4	8.7	9.2	9.3	9.8	10.1	10.5
2	6.8	7.3	7.8	8.5	9.0	9.2	10.2	10.8	11.4
3	6.3	7.0	7.6	8.3	8.9	9.5	10.5	11.2	12.3
4	6.0	6.9	7.5	8.4	8.8	9.5	10.5	11.7	13.0
5	5.9	6.8	7.4	8.0	8.9	9.3	10.6	12.1	13.8
7	5.4	6.6	7.2	8.0	8.8	9.5	10.9	12.6	15.1
9	5.2	6.4	7.0	7.9	8.7	9.6	11.0	13.0	15.6
11	5.0	6.3	7.0	7.9	8.6	9.7	11.4	13.6	17.8
14	4.8	6.2	6.9	7.7	8.6	9.4	11.2	13.8	17.6
17	4.8	6.2	7.0	7.7	8.6	9.6	11.6	15.4	18.2
21	4.7	6.1	6.8	7.7	8.5	9.5	11.6	16.6	18.0
28	4.7	6.1	7.0	7.7	8.6	9.5	11.7	—	17.6
35	4.7	6.1	6.8	7.6	9.1	9.9	12.4	17.0	17.4
42	—	—	—	—	9.0	9.7	—	17.2	17.7

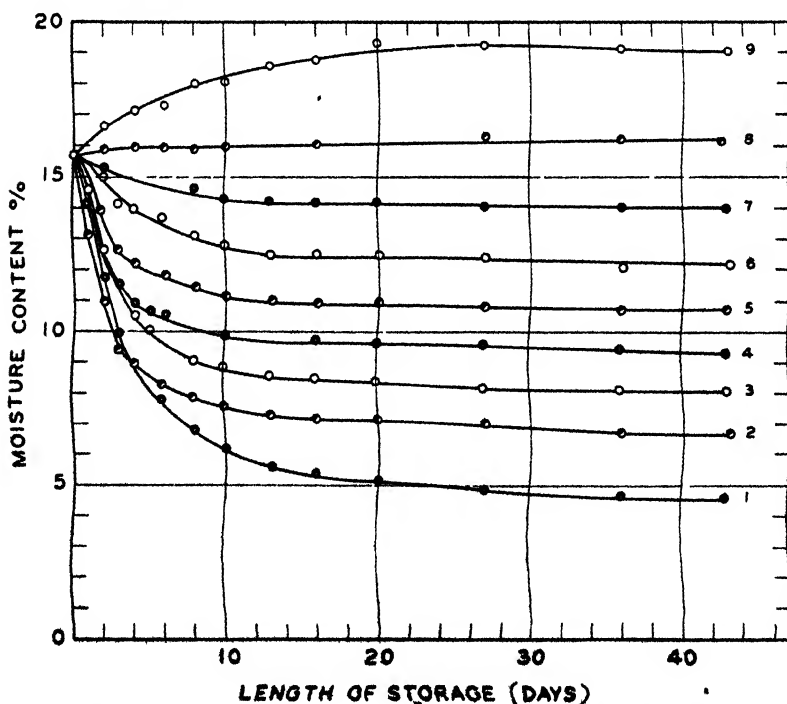


Fig. 1. Effect of length of storage at constant relative humidity on the moisture content of naturally dried rough rice. Relative humidities are: (1) 11.1%; (2) 22.5%; (3) 32.5%; (4) 43.7%; (5) 53.3%; (6) 64.4%; (7) 75.4%; (8) 86.4%; and (9) 92.5%.

TABLE VII

PERCENTAGE OF MOISTURE (WET BASIS) OF HULLS FROM ARTIFICIALLY DRIED ROUGH RICE STORED AT VARIOUS TIME INTERVALS AT CONSTANT RELATIVE HUMIDITY

Conditioning period, days	Relative humidity of conditioning atmosphere, %								
	11.1	22.5	32.5	43.7	53.3	64.4	75.4	86.4	92.5
1	8.4	8.8	9.3	9.8	10.1	10.4	10.5	10.9	10.9
2	7.2	8.0	8.9	9.7	9.8	10.4	10.8	11.2	11.6
3	6.2	7.7	8.5	9.3	9.9	10.4	11.0	11.4	11.9
4	5.9	7.5	8.1	9.0	9.8	10.4	11.0	11.3	12.6
5	5.6	7.0	7.9	8.9	9.7	10.8	11.0	11.6	12.9
7	4.9	6.9	7.6	8.8	9.7	10.5	11.0	12.0	13.5
9	4.5	6.4	7.5	8.7	9.5	10.4	11.0	12.0	13.9
11	4.7	6.4	7.6	8.8	9.7	10.5	11.1	12.2	14.2
14	4.3	6.2	7.4	8.6	9.6	10.4	11.4	12.4	14.6
17	4.2	6.1	7.3	8.4	9.5	10.3	11.1	12.4	14.8
21	4.0	6.1	7.2	8.3	9.6	10.4	11.2	12.6	15.3
28	4.1	5.9	7.2	8.5	9.5	10.4	10.9	—	15.1
35	3.8	5.9	6.9	8.2	9.4	10.4	11.0	12.9	15.1
42	3.8	—	7.0	8.4	—	—	—	12.9	15.2

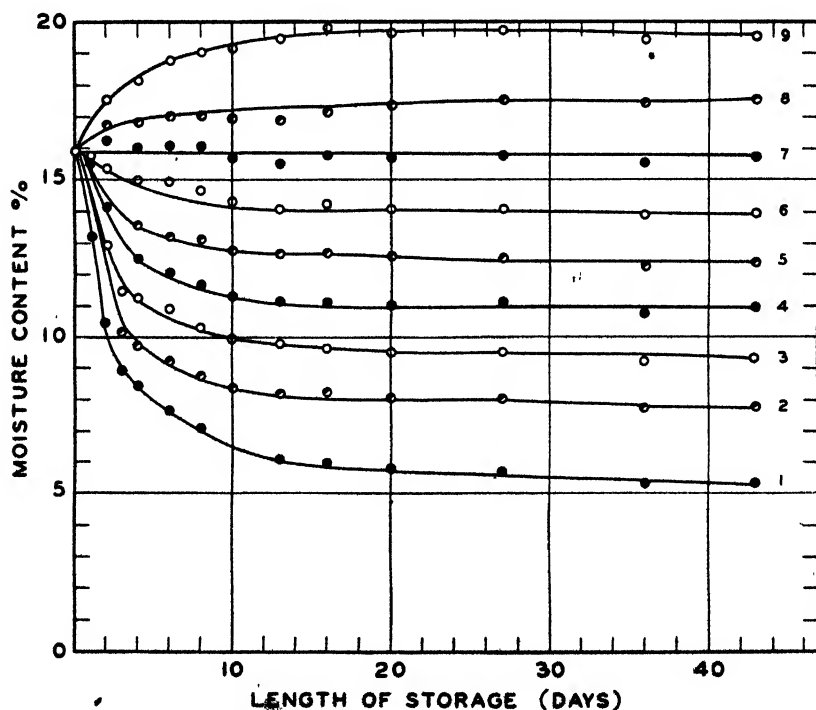


Fig. 2. Effect of length of storage at constant relative humidity on the moisture content of polished rice from naturally dried rough rice. Relative humidities are: (1) 11.1%; (2) 22.5%; (3) 32.5%; (4) 43.7%; (5) 53.3%; (6) 64.4%; (7) 75.4%; (8) 86.4%; and (9) 92.5%.

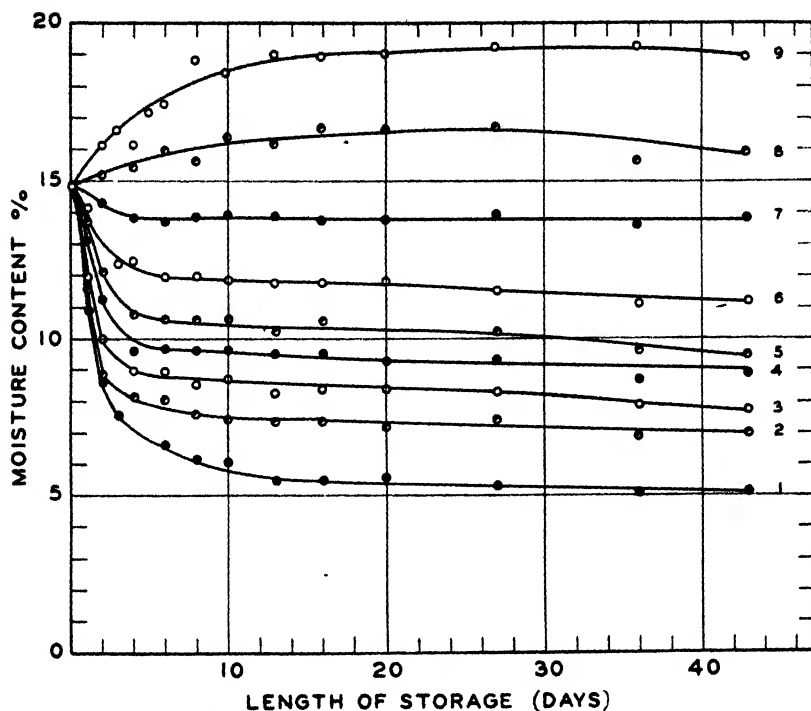


Fig. 3. Effect of length of storage at constant relative humidity on the moisture content of bran from naturally dried rough rice. Relative humidities are: (1) 11.1%; (2) 22.5%; (3) 32.5%; (4) 43.7%; (5) 53.3%; (6) 64.4%; (7) 75.4%; (8) 86.4%; and (9) 92.5%.

TABLE VIII

PERCENTAGE OF MOISTURE (WET BASIS) OF VARIOUS FRACTIONS FROM ARTIFICIALLY DRIED AND NATURALLY DRIED ROUGH RICE IN EQUILIBRIUM WITH ATMOSPHERES OF DIFFERENT RELATIVE HUMIDITIES

Description of sample	Relative humidity of atmosphere, %								
	10	20	30	40	50	60	70	80	90
Rough rice ¹	4.4	6.5	7.9	9.1	10.4	11.8	13.2	14.8	17.6
Rough rice ²	4.6	6.6	8.0	9.3	10.6	11.6	12.6	13.8	17.0
Rough rice ³	—	7.6	9.0	10.2	11.3	12.6	13.8	15.3	18.1
Polished rice ¹	5.2	7.6	9.2	10.5	12.0	13.4	14.8	16.4	18.8
Undermilled ²	4.6	7.0	8.6	10.0	11.4	12.8	14.2	15.4	18.4
Bran ¹	5.0	6.4	8.0	9.0	10.0	11.0	12.4	14.8	18.0
Bran ²	4.6	5.8	6.6	7.4	8.3	9.2	10.6	Moldy	—
Hulls ¹	3.7	5.4	6.8	8.1	9.5	10.8	11.8	12.9	15.3
Hulls ²	3.7	5.4	6.8	7.9	9.1	10.1	10.8	11.6	14.0
Polish ¹	5.3	7.0	8.2	9.2	10.1	11.0	12.4	14.5	18.0

¹ Field-dried rough rice.

² Rice which had been artificially dried prior to receipt at mill.

³ Data of Coleman and Fellows (3) converted to wet basis.

portant consideration in rice drying since, if the outer portion of the rice grain is dried rapidly while the inner part remains relatively wet, strains are set up which produce cracks and checks and subsequently result in many broken kernels during milling. In all cases, however,

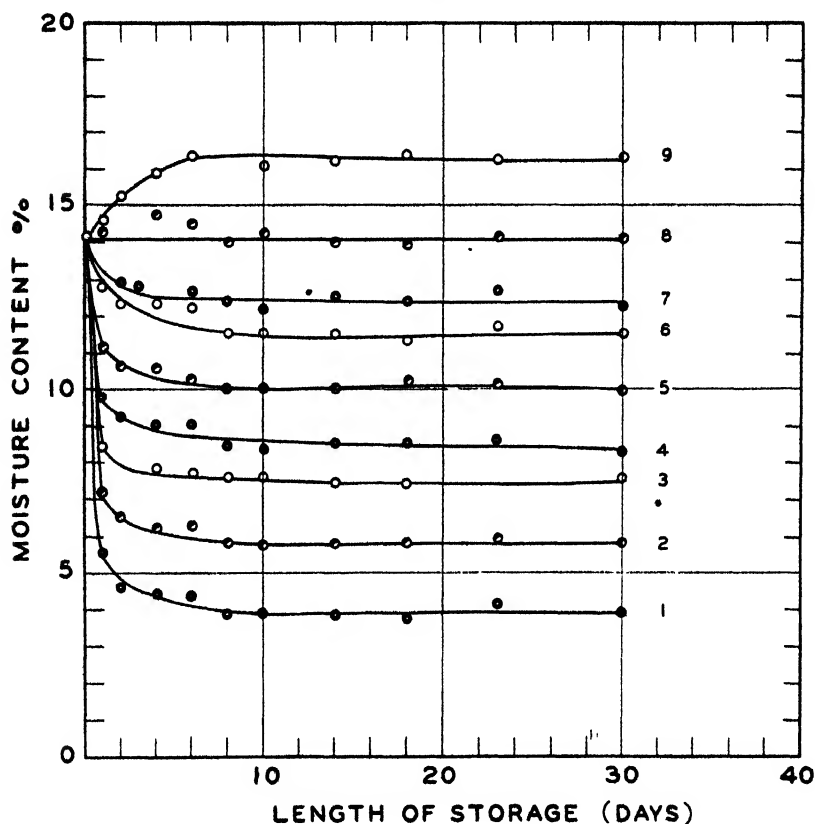


Fig. 4. Effect of length of storage at constant relative humidity on the moisture content of hulls from naturally dried rough rice. Relative humidities are: (1) 11.1%; (2) 22.5%; (3) 32.5%; (4) 43.7%; (5) 53.3%; (6) 64.4%; (7) 75.4%; (8) 86.4%; and (9) 92.5%.

the samples attained hygroscopic equilibrium within a period of three weeks with the exception of those equilibrated over solutions of very high or very low relative humidities.

The equilibrium moisture contents of the samples are given in Table VIII and are shown graphically in Figs. 5 and 6. There were only small differences between the corresponding equilibrium value of naturally-dried and artificially dried fractions. In those cases where

the difference in moisture content at a constant relative humidity is significant, the artificially dried sample contains less moisture.

These data can be considered to be in good agreement with those of Coleman and Fellows (3), who reported equilibrium moisture values for three samples of rough rice approximately 1% higher than those of

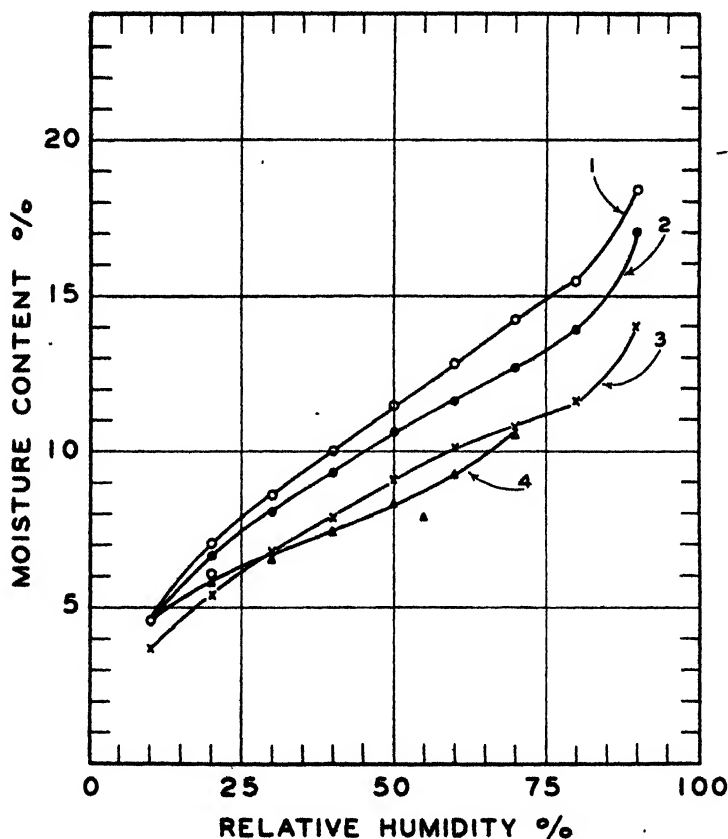


Fig. 5. Moisture content of various fractions of artificially dried rough rice in equilibrium with atmospheres of controlled relative humidity: (1) undermilled; (2) rough rice; (3) hulls; and (4) bran.

the present authors. These higher values are probably explained by the fact that Coleman and Fellows dried their samples at 100°C. in a water oven for 120 hours, whereas the authors used a forced-draft oven for 16 hours. Reference to Table II indicates that in the case of the forced-draft oven method there would be a difference of about 1% in the values obtained with rough rice dried for 16 and 120 hours, respectively.

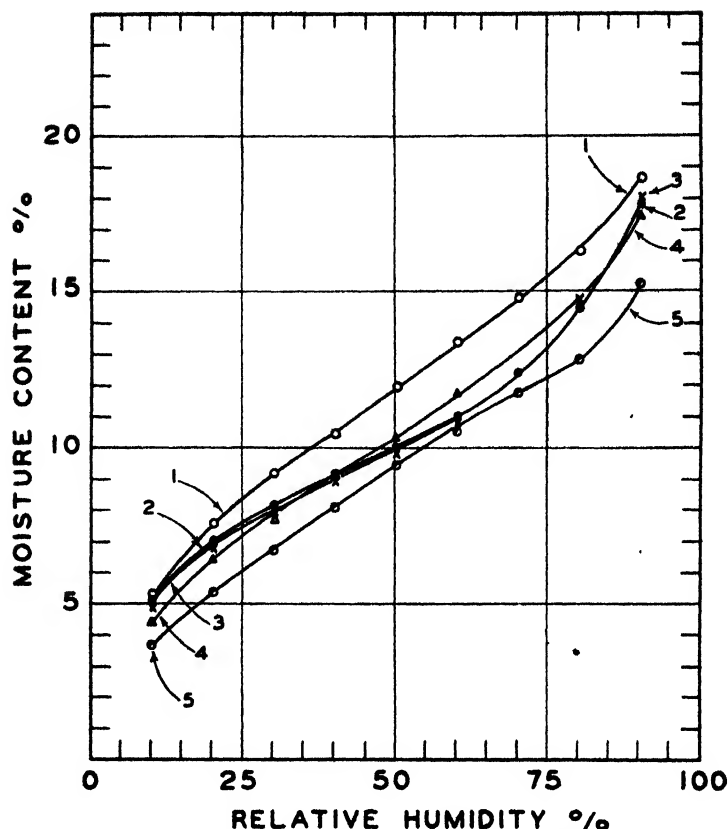


Fig. 6. Moisture content of various fractions of naturally dried rough rice in equilibrium with atmospheres of controlled relative humidity: (1) polished rice; (2) polish; (3) bran; (4) rough rice; and (5) hulls.

Acknowledgment

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INFLUENCE OF GROWTH-REGULATING CHEMICALS ON THE MALTING OF BARLEY AND THE COMPOSITION OF MALT¹

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ABSTRACT

No apparent effect on germination of grain or malt composition was noted when very dilute solutions (0.02–10.0 p.p.m.) of indole acetic acid and naphthalene propionic acid were used in steep or spray water.

In a preliminary malting series using naphthaleneacetic acid and naphthaleneacetamide in concentrations of 2.5–10 p.p.m. as a steeping medium no effect was found in growth or malt composition. In concentrations of 25, 50, 100, and 200 p.p.m. in the steep water, applied to Oderbrucker and Wisconsin Barbless barley, effects were obtained especially at the higher concentrations. The greatest effect was to reduce rootlet development and to increase recovery by approximately 2% with Oderbrucker barley at 200 p.p.m. concentration of naphthaleneacetic acid. Malt extract, diastatic power, alpha-amylase, wort nitrogen/malt nitrogen, and growth were also reduced somewhat by this treatment. The lower concentrations showed less effect on these factors. Wisconsin Barbless was less influenced than Oderbrucker by the treatments. Naphthaleneacetamide treatment resulted in slight increases in malt extract and wort nitrogen/malt nitrogen compared to controls, and in values for rootlet growth and recovery intermediate between the control and the naphthaleneacetic acid treatment.

Three esters of naphthaleneacetic acid that have been used to delay the breaking of dormancy of rose bushes, and that appear to delay hydrolysis of the starch, were applied in vapor form to steeped barley. No marked effect upon the amylase values was apparent although the higher concentration of two esters resulted in increased values for wort nitrogen/malt nitrogen.

During recent years many investigations have been made of the influence of various chemicals on the growth of plants. More studies have been made on phases of plant growth other than germination, and the data are conflicting on the effect of plant-growth-regulating chemicals on this process. Most workers report no effect in very dilute concentrations but an inhibition when higher concentrations are used, or an inhibition at all concentrations (9), (4), (20), (3), (1), (6). Malcher and Zika (12) report that the treatment of barley seed with 0.00625% heteroauxin in most cases gave increased yield of barley and a higher saccharification value of the malt. Crosier *et al.* (5) found that treating seed with dusts containing indolebutyric acid and naphthaleneacetamide increased the percentage of emerged seedlings and total number of heads, especially from shriveled seeds.

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Esters of naphthaleneacetic acid have been used to prevent sprouting of potato tubers, rose bushes, and other plants in storage (6), (14). Hydrolysis of starch is reduced by the treatment, which may indicate inhibition of the amylases. Eyster (8) found that indole-3-propionic, indole-3-butyric, indole-3-acetic, and α -naphthaleneacetic acid reduced diastatic activity *in vitro*. When the diastase was adsorbed on activated charcoal, addition of growth-substances increased diastatic activity over the control.

This paper represents studies carried out over several years to determine the effect of growth-regulating chemicals upon germination during malting and on the composition of the malt. Some of these compounds have also been applied to barley plants in the field at various stages of growth. The effects upon growth, yields per acre, and malting quality will be presented in a later paper.

Materials and Methods

Two varieties of barley, Oderbrucker and Wisconsin Barbless, grown on the University Farm at Madison, Wisconsin, were used. The first series of experiments were carried out on barleys from the 1938 crop, and the later work was done on barleys grown in 1943.

In most of the experiments reported in this paper, the growth-regulating chemicals were applied in the steep water. All barleys were steeped to 45% moisture at 16°C. and malted for 6 days at this temperature and moisture content. The malts were kilned a total of 32 hours according to the schedule: 8 hours, 25°; 4 hours, 35°; 12 hours, 45°; 4 hours, 55°; and 4 hours, 65°C. The rootlet loss and percentage recovery values were obtained by careful weighing before and after cleaning, and all calculations were made on the dry weight basis. The methods of the American Society of Brewing Chemists (2) were used for growth and malt analysis. Growth index as used here represents the sum of values obtained by multiplying the number of kernels in a classification by the higher limit, e.g., no. in $0\text{--}\frac{1}{4} \times \frac{1}{4} + \text{no. in } \frac{1}{4}\text{--}\frac{1}{2} \times \frac{1}{2} + \text{no. in } \frac{1}{2}\text{--}\frac{3}{4} \times \frac{3}{4} + \text{no. in } \frac{3}{4}\text{--}1 \times 1$. Alpha-amylase was determined by the Olson, Evans, and Dickson (16) 20°C. modification of the Sandstedt, Kneen, and Blish method (19), and the values calculated as 20° dextrinizing units (17).

Experimental

Indoleacetic Acid and Naphthalenepropionic Acid in Dilute Solutions. Indoleacetic acid at 10.0 and 0.1 p.p.m. and naphthalenepropionic acid at 2.0 and 0.2 p.p.m. concentration were used for steeping grain of the two barley varieties. One set of samples was steeped 12 hours in solutions of the growth-regulating chemicals and 24 hours

in water. Another set of samples was steeped 36 hours in the growth-regulating solutions. During the malting period the samples were maintained at 45% moisture by spraying with water twice a day.

The summary data for this series are presented in Table I, as averages for the two varieties. It is apparent that there are no significant differences in growth index, recovery, or the three malt compo-

TABLE I

INFLUENCE OF INDOLACETIC ACID AND NAPHTHALENEPROPIONIC ACID AS VERY DILUTE SOLUTIONS IN THE STEEP ON GROWTH AND COMPOSITION OF THE MALTS—AVERAGES OF ODERBRUCKER AND WISCONSIN BARBLESS BARLEYS

Material used	Concentration	Hours steeped in hormone solution	Extract dry basis %	Diastatic power °L.	Wort N. Malt N. %	Growth index	Recovery of malt from barley %
Indole 3 acetic acid	0.1 p.p.m.	12	73.6	152	35.3	81.4	90.2
Indole 3 acetic acid	0.1 p.p.m.	36	73.5	152	34.4	78.6	90.3
Indole 3 acetic acid	10.0 p.p.m.	12	73.6	148	34.8	80.1	90.1
Indole 3 acetic acid	10.0 p.p.m.	36	73.7	146	35.3	80.6	90.3
Water steep		0	73.4	150	34.6	81.8	89.8
Naphthalenepropionic acid	0.02 p.p.m.	12	73.8	154	35.0	81.4	89.9
Naphthalenepropionic acid	0.02 p.p.m.	36	73.6	158	34.4	82.4	91.1
Naphthalenepropionic acid	2.0 p.p.m.	12	73.6	154	34.8	80.1	90.1
Naphthalenepropionic acid	2.0 p.p.m.	36	73.0	145	34.4	80.8	90.3

sition factors that can be attributed to the use of the chemicals. Additional samples of the two barleys were steeped as described above, and in addition indole-3-acetic acid at 100 p.p.m. and naphthalene-propionic acid at 20 p.p.m. were used instead of water as a daily spray to maintain the individual samples at the proper moisture level. Those samples receiving the additional amounts of the two chemicals in higher concentrations showed little difference in growth or malt composition.

Naphthaleneacetic Acid and Naphthaleneacetamide in Several Concentrations. Single maltings were made of Wisconsin Barbless samples from the 1943 crop that had been steeped for 36 hours in solutions containing 2.5, 5.0, and 10.0 p.p.m. of naphthaleneacetic acid, and 5.0 and 10.0 p.p.m. of naphthaleneacetamide. A control steeped in water was malted at the same time. The maximum variations for malt factors between the six malt samples were as follows: extract 0.6%, wort nitrogen/malt nitrogen 2.6%, diastatic power 10°L., alpha-amylase 1.0 20° unit, growth index 7.3, rootlet loss 0.5%, and recovery 0.5%. These values are within the limits of variation between replicate maltings and indicate no effect of the growth substances at these concentrations.

In view of the results obtained above, a more comprehensive experiment was planned using duplicate maltings of two varieties and four concentrations of each chemical, 25.0, 50.0, 100.0, and 200.0 p.p.m. Preliminary tests with the two highest concentrations indicated that germination was not completely inhibited, but that rootlet

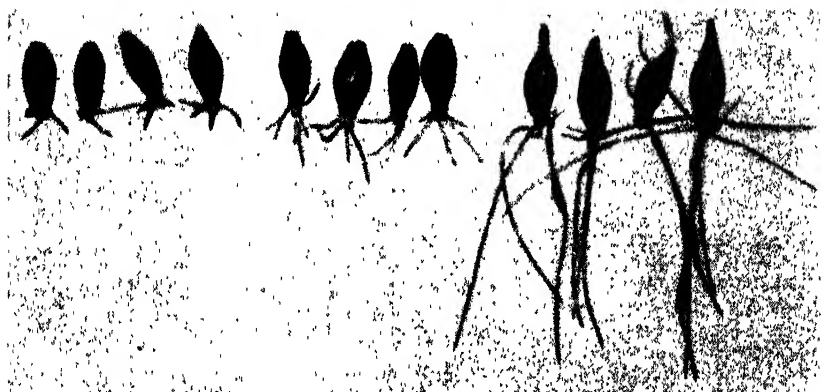


Fig. 1. Effect of naphthaleneacetic acid (200 p.p.m.) and naphthaleneacetamide (200 p.p.m.) on the blotter germination of Oderbrucker barley in comparison with control (right).

development was not normal. Fig. 1 shows a typical effect of 200 p.p.m. of naphthaleneacetic acid and naphthaleneacetamide on rootlet development during germination on blotting paper. Duplicate Oderbrucker samples were steeped 34 hours, and Wisconsin Barbless samples 36 hours in solutions of the two chemicals at the four concentrations. Control samples were steeped in water. The solutions were drained off, and the samples malted as described earlier. The moisture during germination was maintained by spraying the samples with tap water. Fig. 2 shows representative kernels from the treatment with 200 p.p.m. of the chemicals and the control after 4 days germination.



Fig. 2. Effect of naphthaleneacetic acid (200 p.p.m.) and naphthaleneacetamide (200 p.p.m.) on the malting of Oderbrucker barley compared with control water steep after 4 days germination.

The data, as averages of duplicate maltings, for recovery of malt from barley, rootlet loss, extract, wort nitrogen/malt nitrogen, diastatic power, and alpha-amylase are presented as histograms for the individual factors in Figs. 3 to 5.

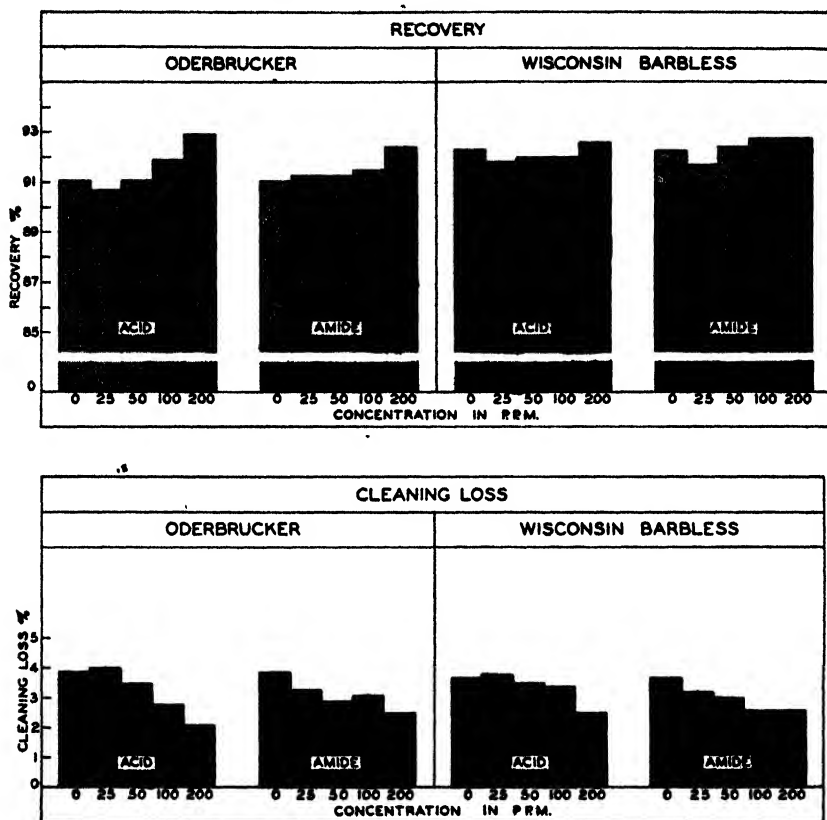


Fig. 3. Effect of naphthaleneacetic acid and naphthaleneacetamide on recovery of malt and cleaning loss.

The detailed data for growth index are not presented, since differences from water steeps are small in relation to variability between duplicate maltings, and are of doubtful significance. The acid treatment resulted in an average growth index of 78, in comparison to 83 for the controls. The amide stimulated growth slightly, producing an average index of 93, which resulted in a significantly different effect of the two growth-regulating chemicals. This effect was similar for the two varieties.

Recovery of malt from barley was increased by the two higher concentrations of both chemicals as indicated in Fig. 3. This increase

was greater with naphthaleneacetic acid than with the amide, and was considerably greater for Oderbrucker than for Wisconsin Barbless. With Oderbrucker, 200 p.p.m. of the acid resulted in an increased recovery of 1.8% over the control; the same concentration of the amide showed an increase of 1.3%. With Wisconsin Barbless, the greatest

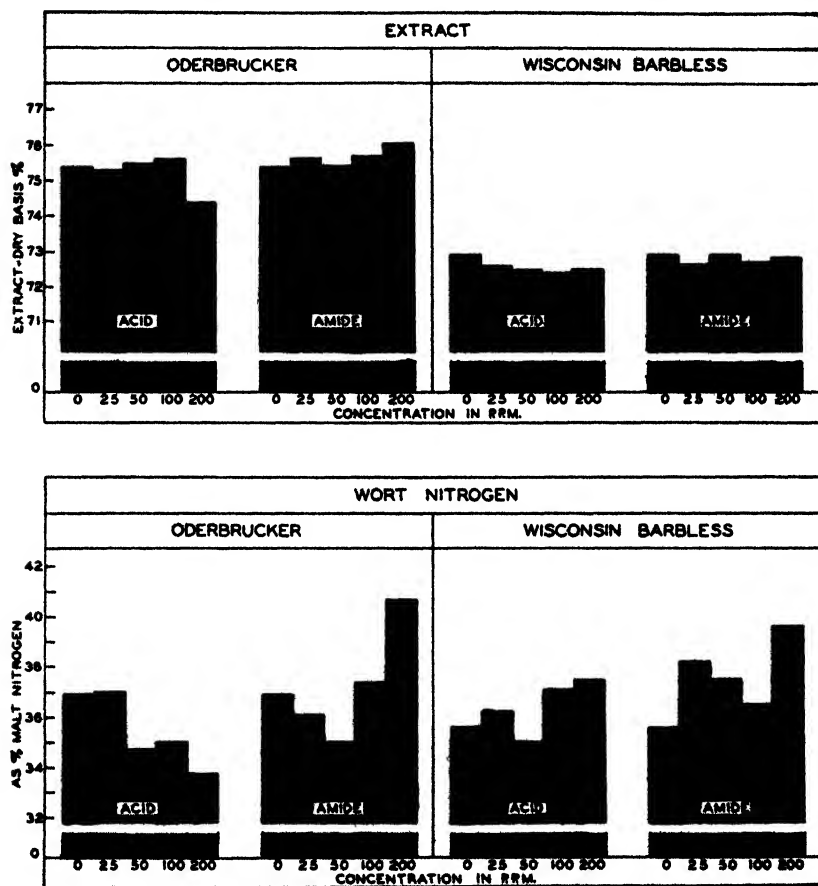


Fig. 4. Effect of naphthaleneacetic acid and naphthaleneacetamide on malt extract and wort nitrogen as per cent of malt nitrogen.

increases over the control were less than 1.0% and were somewhat greater for the amide than the acid.

The recovery of malt from barley is influenced by losses in steep, respiration, and in cleaning. The last consists primarily of rootlets. With Oderbrucker, the reductions in malt cleaning loss by treatment with the two chemicals were as great as the increases in recovery mentioned above. Respiration of Oderbrucker was uninfluenced by

treatment, and recovery increased because of lower rootlet loss. Wisconsin Barbless reacted somewhat differently to treatment. In this variety, respiration was increased slightly by treatment. However, cleaning loss was sufficiently reduced by treatment to result in greater recovery than the control.

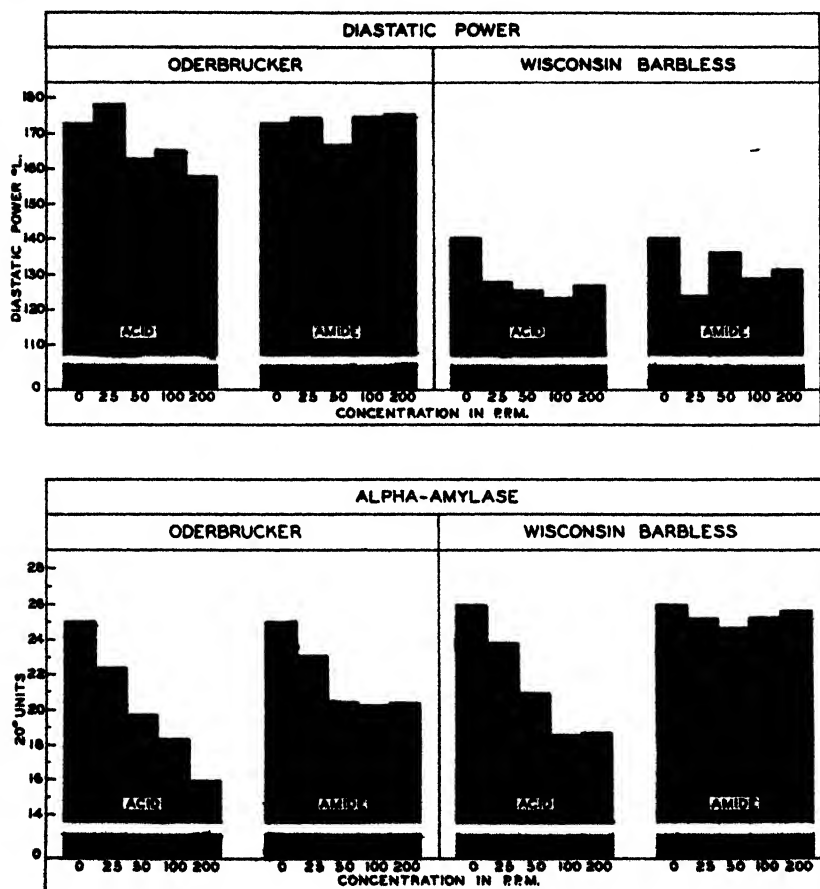


Fig. 5. Effect of naphthaleneacetic acid and naphthaleneacetamide on malt diastatic power and alpha-amylase.

Four quality factors are presented in Figs. 4 and 5 in the discussion of effects of growth-regulating chemicals on malt composition. The two chemicals used produced different effects. Treatment with the acid showed no effect on extract of Oderbrucker malts, but resulted in a small decrease in the Wisconsin Barbless malts. The amide, especially at the 200 p.p.m. concentration, produced increased extract in Oderbrucker malts, but showed no effect on the other variety. Although

significant in relation to the variability between duplicate maltings, the differences in all cases were relatively small, 0.5 to 0.7%.

The wort nitrogen/malt nitrogen ratio has been considered to be a measure of modification in malt, and also a rough measure of proteolytic activity. For this factor also, the chemicals and varieties reacted differently. With Oderbrucker the acid resulted in a small decrease in wort nitrogen at the higher concentrations, but the amide produced an increase, especially at 200 p.p.m. Both chemicals increased the wort nitrogen/malt nitrogen values of Wisconsin Barbless malts, especially at the higher concentrations. The maximum increases over the control samples were obtained at 200 p.p.m. of the amide and were 3.8 for Oderbrucker and 4.0 for Wisconsin Barbless.

Most treatments with growth-regulating chemicals resulted in small but significant reductions in diastatic power values of the malts, as indicated in Fig. 5. An exception was the amide treatment on the Oderbrucker sample which produced no effect. The greatest decreases below the control samples were 15°L. for Oderbrucker samples and 16°L. for Wisconsin Barbless samples, representing decreases of 9 and 11% respectively. These were produced by the highest concentrations of naphthaleneacetic acid.

The effect of treatment on alpha-amylase activity of malts was similar to that on diastatic power, except that the treatment with the amide showed no reduction with Wisconsin Barbless malts. The acid treatment resulted in greater decreases than the amide in the Oderbrucker samples, especially at the higher concentrations. Alpha-amylase activity was depressed more than diastatic power by the treatments. The maximum reductions under the control for Oderbrucker and Wisconsin Barbless were 36 and 28% respectively and were obtained with 200 p.p.m. of the acid.

Volatile Esters Applied as Vapors. Several esters of naphthaleneacetic acid when applied in vapor form have been found to prevent the breaking of dormancy of potatoes and certain shrubs when in common storage. These treatments delay the hydrolysis of starch and the production of reducing sugars. Since this could be an effect on the amylase systems of the plants, it seemed desirable to study, in a preliminary way, the influence of these compounds on malting and malt composition.

Methyl and ethyl esters of naphthaleneacetic acid in concentrations of 0.5 and 2.0 g. per 1,000 cubic feet and 2-methyl naphthylacetic acid at the rate of 2.0 g. per 1,000 cubic feet were vaporized and applied to duplicate Oderbrucker barley samples which had been steeped previously to 45% moisture. The calculated quantities of the esters

were dissolved in 5 ml. of absolute alcohol and this poured onto the bottoms of 2-gallon friction top pails. The steeped barleys were placed in the pails, the lids placed on tightly, and the chemicals volatilized by placing the pail on a hot plate for 5 minutes. The pails were then placed in a 16° chamber and the steeped barleys allowed to stand in contact with the vapors for 12 hours. At the end of this period the samples were removed from the pails and malted for 5½ days at 16°C. and 45% moisture. The control samples were given the same treatment except that 5 ml. of absolute alcohol only were used. The data for growth, recovery, and certain of the malt factors as means of duplicate maltings are presented in Table II. The effects of all

TABLE II

INFLUENCE OF VOLATILIZED ESTERS OF NAPHTHALENEACETIC ACID APPLIED TO STEEPED ODERBRUCKER BARLEY ON THE GERMINATION AND COMPOSITION OF THE MALT

Materials used	Concentration g. per 1000 cu. ft.	Growth index	Re- covery of malt from barley %	Loss in cleaning malt %	Ex- tract dry basis %	Wort N. Malt N. %	Dia- static power °L.	Alpha- amylase 20° dext. units
Ethyl ester naphthaleneacetic acid	0.5	79.7	91.8	3.5	75.9	36.5	170	24.9
Ethyl ester naphthaleneacetic acid	2.0	84.4	91.1	3.8	76.3	37.5	178	25.0
Methyl ester naphthaleneacetic acid	0.5	83.9	91.9	3.2	75.1	37.5	171	24.8
Methyl ester naphthaleneacetic acid	2.0	78.2	92.0	3.3	75.3	41.8	168	23.2
2-Methyl-1-naphthylacetic acid	2.0	76.9	91.9	3.3	75.5	36.1	167	23.6
Control	0.0	80.9	91.9	3.4	75.5	35.7	167	24.2

treatments were small and only a few were significant. The higher concentration of the ethyl ester produced the highest growth index and rootlet loss, with the concomitant low recovery; the highest value for extract, diastatic power, and alpha-amylase, and a relatively high value for wort nitrogen/malt nitrogen. All treatments resulted in increased values for wort nitrogen, but only the higher concentration of the methyl ester gave an appreciable increase. Further studies with the esters on more samples would be required to verify these results.

Discussion

Although the literature dealing with the effect of growth-regulating chemicals on germination and growth of cereals is somewhat conflicting, most studies have shown no stimulation of germination at very low concentrations, but inhibition or retardation at higher concentrations. Marmer (13), Weimer (20), and others present evidence that germinat-

ing seeds themselves contain relatively large quantities of auxins or heteroauxins. The results obtained on malting of barley with several different chemicals are in agreement with these findings. The retardation of barley germination and growth appeared to be confined primarily to rootlet development, with only slight effects on elongation of the plumule or acrospire. This is in agreement with the reaction of wheat seedlings to 100–200 p.p.m. of indole-3-acetic acid as found by Loo and Tang (11) and others. Among the explanations given for the effect of these compounds is an increase in the respiration of wheat seedlings associated with an inhibition of growth by 50 p.p.m. of indole-3-acetic acid (18). Hsueh and Lou (10), on the other hand, found that high concentrations (0.1%) of 2-4 D inhibited aerobic respiration and germination of barley. The rough measures of respiration used in this study indicated either no effect or a slight stimulation of respiration of barley during malting by naphthaleneacetic acid and naphthaleneacetamide.

Eyster (8) and Miller and Kneen (15) report an inhibition of amylase action by growth-regulating chemicals *in vitro*. The decreases in amylase activity obtained in this study are probably due to physiological effects upon the germination process rather than the effect of small residual quantities of the chemicals remaining on the malt.

Preliminary experiments with those esters of naphthaleneacetic acid that delay the breaking of dormancy of tubers and shrubs in storage indicated no effect on the germination or amylase activity of barley malts. Denny, Guthrie, and Thornton (6) reported an inhibition of the hydrolysis of starch and production of reducing substances by these materials when they were applied to potato tubers. This effect, possibly upon the amylases, may be an indirect one of controlling physiological processes leading to the hydrolysis.

The effects of the higher concentrations of naphthaleneacetic acid and naphthaleneacetamide upon germination, while primarily of interest in relation to physiology of germination, indicate certain practical applications in malting. By the choice of chemical and concentration, it should be possible to increase recovery of malt from barley by 1 to 2% without excessive deleterious effects upon the malt characteristics. There is some evidence that one might improve malt quality over a water-steeped sample, and still obtain an improved recovery of approximately 1%. Plant scale tests of the use of growth-regulating chemicals would be required to determine the practicability of their commercial use, if not undesirable for other reasons. These data at least indicate the desirability of investigating other chemicals and concentrations applied to more barley varieties.

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SIGNIFICANCE OF LOAD-EXTENSION TESTS IN ASSESSING THE BAKING QUALITY OF WHEAT FLOUR DOUGHS¹

P. HALTON²

ABSTRACT

An apparatus is described with which the rate of extrusion of dough through an orifice under constant pressure can be measured. With it the absorptions of different flours can be assessed by determining the water contents necessary to give doughs having the same rate of extrusion.

An extensometer is described with which load-extension curves can be made on dough.

Doughs made either with or without yeast soften with age and it is necessary to allow for this in comparative baking or load-extension tests. This softening, which is shown to be equivalent to an increase in the water content of the dough, can be compensated for by adjusting the water contents of doughs made from different flours so that they all have the same extrusion time at the period when they are baked or tested on the extensometer.

As a result of this softening the force required to stretch an unyeasted dough decreases with increasing age of the dough. With a yeasted dough the reverse occurs and the stretching force increases. It is suggested that this is not due to a tightening of the dough during fermentation but to modifications in its chemical and physical condition which result in increased work hardening of the dough when stretched. The fermenting dough decreases in extensibility and at the same time the relaxation time increases. It is this change in the relaxation time which is the basis of dough ripening and improved baking quality. It is shown, in this connection, that the fermentation necessary for a given decrease in extensibility and the fermentation time necessary for optimum quality bread bear similar relationships to the yeast content of the dough.

Experimental evidence is presented to show that bread quality is a function of the tensile strength and of the relaxation time of the dough. The tensile strength of the dough is shown to be the basis of flour strength and determines the breadmaking potentialities of the flour, i.e., the quality of bread that can be obtained under optimum conditions. The relaxation time of the dough, on the other hand, is a measure of the condition of the dough and thus largely determines the quality of bread the dough will actually produce. The tensile strength of dough is a function of the wheat from which the flour was milled and does not appear to be affected by normal fermentation, improver treatment, or mechanical manipulation of the dough. On the other hand, the relaxation time is considerably modified by the various chemical and physical processes which form the basis of bread-making.

Both the tensile stress and the relaxation time of dough can be assessed from a load-extension test.

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A wheat flour dough has certain characteristics normally associated with solids, but in other respects it resembles a liquid. When, for example, a piece of dough is stretched and then released it shows the elastic recovery of a solid. If stretched to any appreciable extent, however, this recovery is not complete and a permanent change in shape results. This nonrecoverable stretch is due to the dough having flowed like a liquid. This fluid nature of dough is also seen when a piece is left to rest on a board and flows out under its own weight.

Halton and Scott Blair (6) studied this dual nature of dough by stretching, under constant force, cylindrical test pieces floated on a bath of mercury. When the stretching force had been applied for a given time the increase in length of the dough cylinder was measured. The applied force was then removed to allow elastic contraction to take place. By this means the total stretch was divided into its two components, the elastic or recoverable stretch and the viscous or non-recoverable stretch. Such tests formed the basis for determining the rigidity modulus and the viscosity of dough.

From experiments made with flours of varying baking quality it was found that the viscosity and modulus values depended on a number of factors including the water content and age of the dough. In addition the following two generalizations were arrived at: 1. When the correct absorptions, found for each flour from baking trials, were used, doughs made with various flours differed considerably in their viscosities but had similar values for modulus. This indicated that measurements of rigidity modulus might be used for assessing the water absorption of different flours. 2. When data obtained on a number of flours were examined it was found that the better the breadmaking quality of the flour the higher was the ratio of the viscosity to the rigidity modulus of the dough. The authors explained how this ratio, which is equivalent to Maxwell's "relaxation time," (9) determined the "spring" of "elasticity" of the dough, a factor which bakehouse experience had shown to be closely related to baking quality.

Halton and Scott Blair also investigated the connection between the physical properties of dough and its "ductility" or "extensibility," another factor which bakehouse experience had shown to be related to baking quality.

When the viscosity of dough is determined by a stretching test the actual value obtained depends on the stress applied to the dough and also on the strain to which the dough is subjected. When the stress is increased the viscosity falls, a phenomenon known as "structural viscosity." On the other hand, when the strain is increased the viscosity rises, the dough exhibiting the characteristic of "work hardening" shown by some metals. If the applied force remains constant

during stretching, the stress and strain may both increase, the stress, i.e., force per unit cross section, increasing due to the thinning of the test sample. When both stress and strain increase, the viscosity of the dough is modified by both work-hardening and structural viscosity.

Halton and Scott Blair obtained indications that variations in structural viscosity were associated with variations in the extensibilities of different doughs. Experiments showed, however, that this only applied to some flours and that measurements of structural viscosity, as then made, did not give a true indication of dough extensibility in all cases. It was realized that one possible reason for this was that, with the technique used, the stresses and strains were much smaller than those operating in a fermenting dough under conditions which lead to tearing and rupture. The present paper deals with the continuation of this work in which the modified technique of Schofield and Scott Blair (11), more suitable for the study of dough properties at large stresses and strains, has been used.

The viscosity and rigidity modulus of dough can be calculated from the equations:

$$\text{viscosity} = \frac{\text{stress}}{\text{viscous strain in unit time}}$$

$$\text{rigidity modulus} = \frac{\text{stress}}{\text{elastic strain}}$$

In these equations three factors are involved, i.e., stress, strain, and time. In the technique used by Halton and Scott Blair, stress and time of application of the stress were kept constant while variations in strain were measured. In the modified technique used in the experiments now to be described, strain and time were kept constant while variations in stress were measured.

Materials and Methods

The apparatus used (see Fig. 1) consisted of a mercury bath on which a cylinder of dough, approximately 0.7 cm. in diameter, was floated. One end of the dough was fastened to a piece of cork connected by a cotton thread to a winch operated by a constant speed

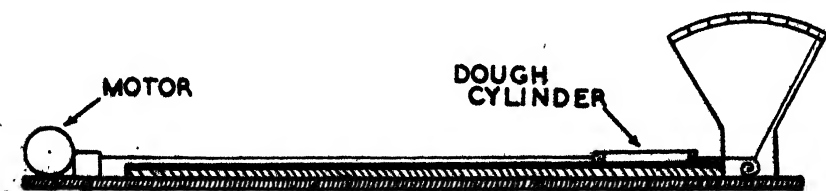


Fig. 1. Mercury bath extensometer—diagrammatic.

motor. The other end of the dough was fastened to a second piece of cork connected by a cotton thread to a load meter. This meter contained a coiled spring to which was attached a pointer operating over a graduated scale. When the motor was started, the dough cylinder stretched, the increase in length being proportional to the time of stretching less a small correction due to the shift in position of that end of the dough attached to the load meter. The force built up during stretching was read off at intervals on the scale of the load meter, one unit on the scale being approximately equivalent to 1 g. From the data so obtained load-extension curves such as is shown in Fig. 2

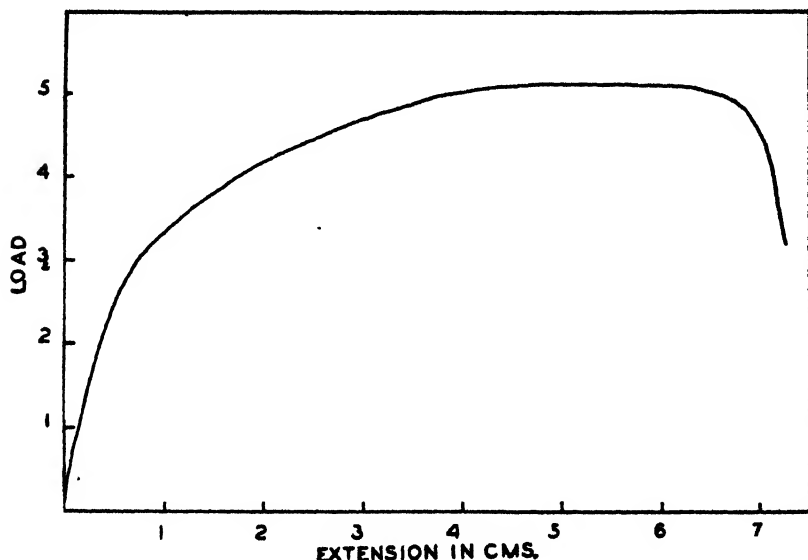


Fig. 2. Load-extension diagram obtained on mercury bath extensometer.

could be obtained. Such curves were, however, of little value since their dimensions depended upon the water content of the dough and no differentiation between the viscous and elastic deformations was possible. In practice, therefore, the dough was stretched for a given time, the direction of the motor was then reversed to allow the dough to relax, and when this was complete and the load meter reading had returned to zero, the motor was once more put in its forward position and the stretching of the dough continued until rupture occurred. By this procedure data were obtained from which curves, such as is shown in Fig. 3, were constructed.

This curve shows the change in load and extension during the three stages of the test, A-B being the first extension, B-C the relaxation,

and C-D the final stretch ending at the point D when the dough rapidly tore and then broke.

At the time corresponding to point B in the curve the dough cylinder had stretched 1.25 cm., this being partly elastic and partly viscous. On relaxation an elastic recovery of 0.75 cm. took place leaving a permanent elongation of 0.5 cm., this last being a measure of the viscous flow. By this separation of the dough extension into its two components the viscosity and modulus of the dough could be calculated. The actual values in this particular case were viscosity = 0.51×10^6 and modulus = 0.88×10^4 C.G.S. units.

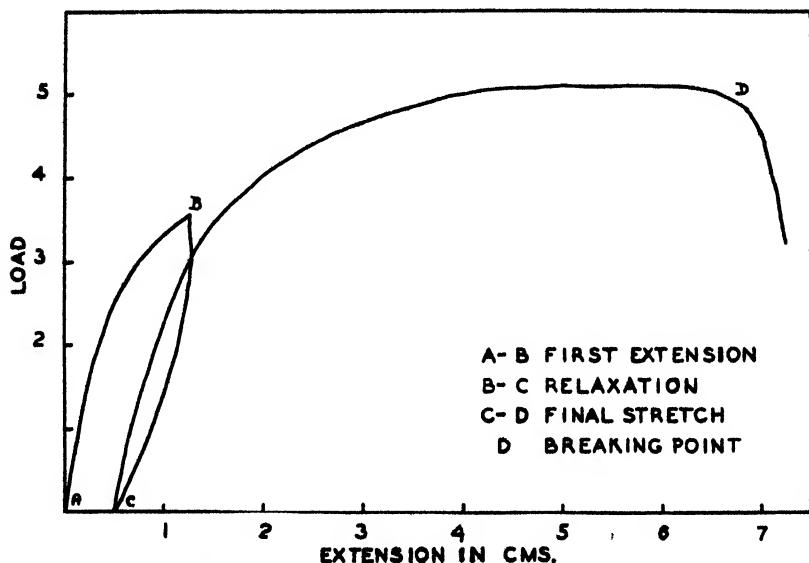


Fig. 3. Load-extension diagram with relaxation of dough.

It will be realized that these values applied to the dough during the stretching from A-B. By relaxing the dough after different periods of stretching, however, the change in the physical properties throughout the stretching period could be assessed and thus the over-all effect of work hardening and structural viscosity determined.

When load-extension curves had been obtained on a number of flours a different use for these curves became apparent. It has been pointed out earlier in this paper that when the physical properties of doughs made from different flours are to be compared, the water contents should be so adjusted that the doughs all have a similar modulus. This adjustment was made for the load-extension tests and an examination of the resultant curves showed that in general the better the baking quality of the flour the greater was the height of the curve.

In other words the load developed during stretching was an indication of the breadmaking quality of the dough.

The reason for this is that the force required to stretch a dough depends on both its viscosity and its modulus. If a number of doughs having similar values for modulus are tested, any differences in their viscosities will therefore be reflected in corresponding differences in the force required to stretch them. These variations will therefore



Fig. 4 Self-recording extensometer.

run parallel with variations in the viscosity-rigidity modulus ratio or relaxation time of the dough and, from the work of Halton and Scott Blair, will therefore correlate with variations in baking quality.

The significance of this finding lay in the possibility that a single load-extension test might give a measure of two important dough properties: the load giving an assessment of the elasticity of the dough and the extension an assessment of the ease with which the dough tore when stretched.

The examination of load-extension curves for a number of flours showed that while variations in the heights of the curves agreed with what was to be expected from baking tests on the flours, the length of the curves did not correlate well with the extensibilities of the doughs as assessed in the bakehouse. Indications were obtained that this might be due to the method of preparing the dough cylinders by extrusion. Trials were therefore made with balls of yeasted dough which could be

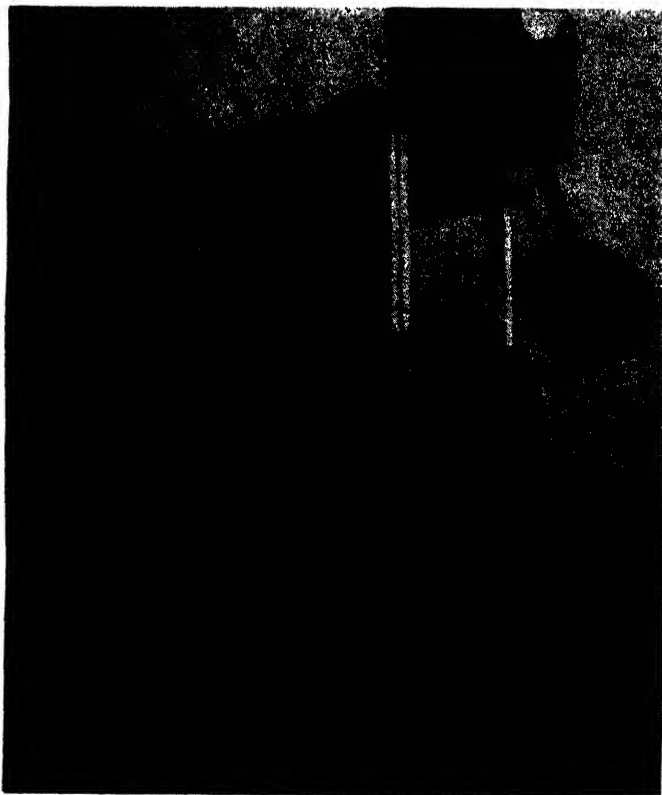


Fig. 5. Self-recording extensometer.

handled much the same as the larger bakehouse doughs. Experience soon showed that a much truer measure of extensibility was being obtained and in view of this the simple extensometer (5) shown in Figs. 4 and 5 was constructed to enable load-extension tests to be made on this shape of test sample.

The ball of dough is impinged on two semicircular pegs which lie in contact. These pegs are each attached to a crosshead which is positioned by and can slide up and down two vertical guides. The

crosshead of the top peg is attached to a balance while the crosshead of the bottom peg is attached to a threaded sleeve which is moved up or down by a threaded rod activated by an electric motor. This rod also operates through gearing to turn a recording drum. The upper crosshead engages one end of a pivoted arm, on the other end of which is a pen which presses against the recording drum.

When the dough has been impinged on the two pegs the motor is switched on. The bottom peg then moves downwards at constant rate and the recording drum revolves. As the dough is stretched the force built up pulls the top peg downwards against the opposing balance weight, the degree of movement being proportional to the force. This movement is recorded and magnified by the upward movement of the pen writing on the chart wrapped round the recording drum. When the dough has been extended to the point when it breaks, the force pulling on the top dough peg is released and as this peg moves upwards, the recording pen completes the load-extension curve drawn on the chart.

This machine, like the Chopin Alveograph (1) and the Brabender Extensograph (10), is only suitable for comparative tests on different doughs. Actual measurements of dough viscosity and elasticity cannot be determined from the curves. Because of this some method other than the direct measure of the rigidity modulus had to be used for assessing the water contents at which doughs made from different flours should be compared.

TABLE I
VISCOSITY AND RIGIDITY MODULUS OF DOUGHS HAVING
SAME EXTRUSION TIME

Flour	Viscosity	Modulus
No.	C.G.S. units	C.G.S. units
1	0.7×10^6	1.0×10^4
2	0.5×10^6	0.9×10^4
3	0.4×10^6	1.0×10^4
4	0.4×10^6	1.0×10^4
5	0.3×10^6	1.0×10^4
6	0.2×10^6	1.0×10^4

The method adopted was to fix these water contents so that all doughs had the same rate of extrusion through an orifice, a method previously used by Jago (7) (and later by Stamberg and Bailey, 12) for determining the water absorptions of different flours. In the work of Halton and Scott Blair (6) in which dough cylinders were prepared by extrusion from a dough gun it was thought that the rate of extrusion was determined by the viscosity of the dough. Later experiments, however, showed that the rate of extrusion was dependent more

on the modulus than on the viscosity. This is illustrated by the data given in Table I. These figures were obtained on doughs made from different flours, the water content in each case being adjusted so that all the doughs had the same rate of extrusion. It will be seen that although the viscosities of the doughs varied considerably there was little variation in their rigidity moduli.

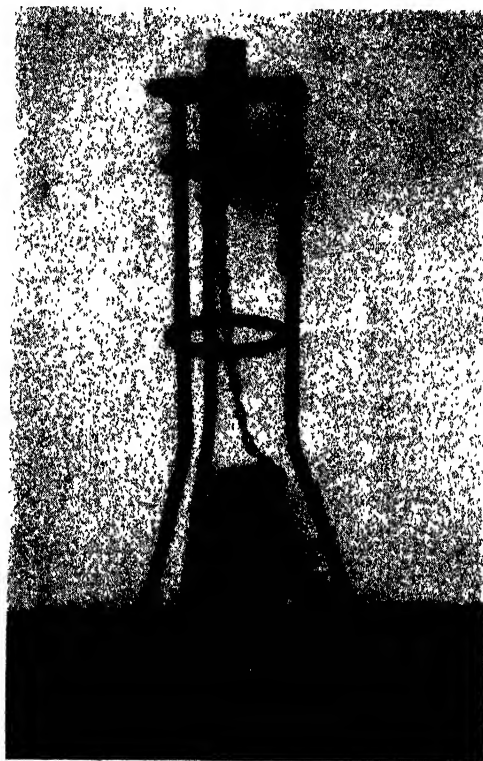


Fig. 6. Water absorption meter.

From these findings it followed that a constant rate of extrusion could be used for assessing the water content at which a dough should be examined on the extensometer.

The dough gun constructed for determining the water absorptions of flours is shown in Figs. 6 and 7. It consists of a hollow cylinder closed at one end by a plate in the center of which is a hole through which the dough is extruded. The cylinder passes through the center of a circular plate resting on leveling screws threaded on three supporting up-rihts. The dough is forced out of the cylinder by a piston attached to a rod which passes through the dough and through the

orifice. The bottom of this rod is connected by a pin to a collar to which is also attached a chain carrying a weight. The pin forms a pointer which moves over the scale attached to one of the supporting uprights.

The dough to be tested is put into the cylinder of the dough gun. The piston is placed on top and the weight attached to the piston rod.



Fig. 7. Water absorption meter.

The dough is extruded through the annular space formed by the piston rod passing through the orifice in the bottom plate of the cylinder. The time for the pointer to move over the scale is measured.

The logarithm of the extrusion time is lineally related to the water content of the dough. Based on this finding a special slide rule was constructed, the time scale of which is logarithmic in form. When the extrusion time for a dough of known water content has been measured, the absorption corresponding to the standard extrusion time is calculated by the use of the slide rule.

The water absorption, corresponding to a constant rate of extrusion, is used in making the doughs to be stretched on the extensometer.

For these tests a parent dough is made from 280 g. flour. This is scaled off into 75-g. pieces which are left to rest under glass jars. Twenty-five minutes before they are tested the doughs are passed once through the molder shown in Fig. 8. This machine is a modified

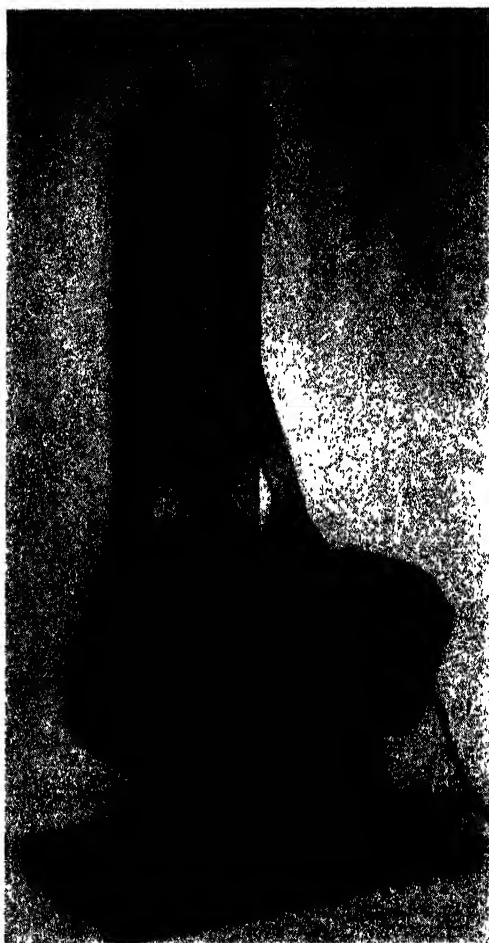


Fig. 8. Dough molder.

version of the punching machine described by Malloch and Hopkins (8). It consists of a framework supporting rollers over which runs an endless vertical belt driven by an electric motor. On one side of the belt is a pressure plate which keeps the belt in contact with a wooden tunnel. This is of semicircular cross section decreasing in radius from the top to the bottom.

At the end of the 25-minute relaxation period after molding the test doughs are stretched on the extensometer. While experiments have been made on yeasted and nonyeasted doughs, the data obtained on the former are generally of more value for interpreting the importance of the physical properties of dough in determining baking quality. Some of the factors involved and their effect on the extensometer curves and on baking quality will now be considered.

Discussion

Water Absorption. A correct measure of the water absorption of a flour is essential if a true assessment of its quality is to be obtained by baking or physical dough tests. When determining this absorption, however, allowance must be made for the fact that during the period between making and baking or testing, the dough may soften.

Halton (4) showed that during this period the fall in the viscosity and rigidity modulus of the dough and, what is of greater significance, the change in the ratio of their values were the same as those resulting from an increase in the water content of the dough. Thus after resting the dough had, in effect, a higher free water content than when freshly made, a change similar to that resulting from syneresis. This view of the softening of dough with age is supported by the work of Baker, Parker, and Mize (2) on the liquors obtained by subjecting doughs to the action of a Sharples supercentrifuge. The amount of liquor they obtained increased when the dough rested after making. They also found that this increase was greater for yeasted than non-yeasted doughs which is in keeping with the author's finding that the softening as measured by the fall in the extrusion time is greater when the dough contains yeast.

The physical condition of the dough and therefore the quality of the loaf it will give is modified by this softening. It is therefore necessary to allow for it, which can be done by determining the extrusion time of the dough, not when it is freshly made but at a time corresponding to that at which the dough would be put into the oven or tested by physical means. The procedure used by the author is to make two or three small doughs from the same flour but with different water contents and to leave these to stand for the necessary time before measuring their extrusion times. The doughs are made with or without yeast, depending on the information required. The points relating the water contents of the doughs when made to the logarithms of their extrusion times lie on a straight line from which the water content necessary to give a dough having the standard rate of extrusion can be read off. By this means the water contents of all doughs, whether made for physical or baking tests, are adjusted so

that when stretched on the extensometer or baked they have the same extrusion time.

The degree of softening of a dough with age, as shown by the fall in its extrusion time, depends on a number of factors including the variety and quality of the wheat from which the flour was milled as well as the percentage extraction of the flour and its content of damaged starch. A dough made from a low extraction flour milled from a strong, sound grist may soften by the equivalent of only 0.5% in absorption during 3 hours' fermentation. During the same time a dough made from a flour of 85% extraction milled from a medium strength grist may soften by the equivalent of 7% in absorption.

Fermentation. Data from extensometer curves obtained on unyeasted doughs of different ages but made from the same flour are given in Table II. It will be seen that as the time between making and

TABLE II
EFFECT OF AGE OF UNYEASTED DOUGHS ON EXTENSOMETER
CURVES AND EXTRUSION TIME

Age	Height	Length	Product	Extrusion time
<i>Hour</i>	<i>cm.</i>	<i>cm.</i>		<i>Secs.</i>
1	2.9	24	70	92
2	2.6	25	64	84
3	2.3	27	62	76

testing the dough increased, the height of the curve decreased, its length increased, and the product figure of height \times length decreased.³ These changes in the shape of the curve (like the fall in the extrusion time) suggest a softening of the dough similar to that which results from increasing its water content.

If the change in dough with age, as reflected by the change in the extensometer curve, were entirely due to an increase in its free water content, it should be possible to compensate for it by a suitable reduction in the water added to the dough when made. To test this point a number of doughs were made from the same flour but with varying water contents. The extrusion times of the doughs were measured when they were 1, 3, and 5 hours old and from the data so obtained the water contents necessary to give doughs having the same extrusion time when 1, 3, or 5 hours old were determined. Thus when freshly made the 5-hour dough had the lowest water content and the highest

³ This applies to doughs made from flours treated with nitrogen trichloride or ammonium persulfate as well as untreated flours. With bromated flours, however, the height of the curve may rise despite the fact that the extrusion time of the dough decreased. This difference in the behavior of bromated doughs is undoubtedly due to the fact that the improving action of potassium bromate is progressive with time. As a consequence, the relaxation time of the dough will tend to increase progressively due to the increasing effect of the bromate and this may more than offset the effect due to the progressive softening of the dough. This time factor in the bromate effect is shown up by the work of Munz and Brabner (10) on the relaxation of dough after molding.

extrusion time while the 1-hour dough had the highest water content and the lowest extrusion time. After standing for their appropriate times each dough had the same extrusion time and, in addition, when stretched on the extensometer they gave similar curves. These tests indicate, therefore, that the changes in the extensometer curves, obtained on a nonyeasted dough as it ages, reflects a softening due to an increase in the free water content of the dough. The degree of softening and the increase in free water can be determined from the change in the extrusion time of the dough.

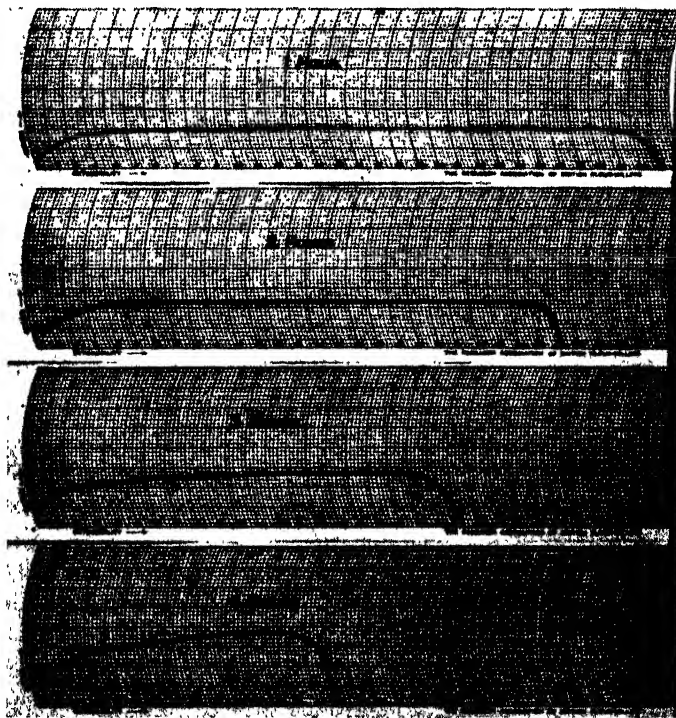


Fig. 9. Effect of fermentation of dough on extensometer curve.

Extensometer curves on yeasted doughs fermented for 1, 2, 3, and 4 hours are shown in Fig. 9. It will be seen that with increasing age of the dough the height of the curve increased and its length decreased, the opposite to what occurred with nonyeasted doughs. This might be taken to indicate that the yeasted doughs became tighter and not softer with age. On the other hand, the data for these doughs given in Table III show that the product figure (height \times length of curve) and the extrusion time both decreased with increasing age, suggesting that the dough softened.

When interpreting the results of physical tests on dough it is necessary to bear in mind that the technique of the test may modify the physical characteristics of the dough. This is certainly true of stretching tests which normally work harden and thus toughen the dough. As a consequence the shape of the extensometer curve depends both on the condition of the dough before it is stretched and also on its response to stretching. The extrusion times for both yeasted and non-yeasted doughs fall as the doughs age, suggesting that both soften.

TABLE III
EFFECT OF AGE OF YEASTED DOUGHS ON EXTENSOMETER
CURVES AND EXTRUSION TIME

Age	Height	Length	Product	Extrusion time
<i>Hour</i>	<i>cm.</i>	<i>cm.</i>		<i>Secs.</i>
1	2.0	30	60	90
2	2.3	26	59	78
3	2.7	21	57	72
4	3.7	15	55	69

Bakehouse experience also suggests that doughs when left undisturbed, i.e., not molded or stretched, soften. On the other hand, when molded they toughen, so their final characteristics depend on the balance of the two processes. The difference in the yeasted and nonyeasted dough, as reflected by the difference in their extensometer curves, can be explained by assuming that the nonyeasted dough toughens when stretched to the same or decreasing extent with increasing age, whereas the yeasted dough work hardens to an increasing extent as fermentation proceeds.

If a series of, say, four yeasted doughs have their water contents adjusted so that when they are 1, 2, 3, and 4 hours old, respectively, they have similar extrusion times, the corresponding extensometer curves are not identical, as with nonyeasted doughs, but show a progressive increase in height and decrease in length with increasing fermentation. On the other hand, the product figure does remain constant, indicating that the fall in the value of this figure with increasing age reflects a softening of the dough. As with nonyeasted doughs, the degree of softening can be assessed, from the change in extrusion time, in terms of an increase in water content.

While the difference in the aging characteristics of yeasted and unyeasted doughs may be due, at least in part, to the chemical changes that accompany fermentation and which modify the oxidation-reduction systems in the dough, they can also be explained in terms of the physical history of the dough. The unyeasted dough remains static during the rest period between making and stretching. The

yeasted dough, on the other hand, is subjected to stresses resulting from the gas generated during fermentation and which stretches it. The stretching proceeds at a slow rate compared with the stretching on the extensometer or that due to molding and this allows time for the relaxation due to viscous flow to keep pace with the stretching. In consequence the dough does not work harden as when stretched at a much more rapid rate. Despite this, important changes may occur in the dough. Links in the protein network may be broken, the weaker links giving first so that as fermentation proceeds only the

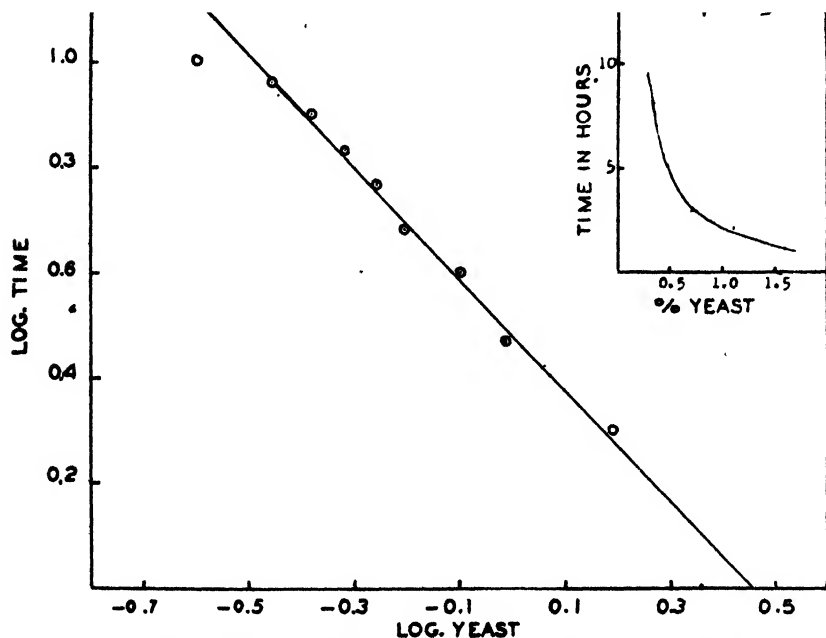


Fig. 10. Relation between yeast content of dough and fermentation time for best loaf.

stronger links remain attached. Thus as fermentation continues the dough work hardens to an increasing extent when it is stretched, this being reflected in extensometer curves of increasing height. As more and more of the links are broken the dough becomes more brittle and less extensible; thus the increase in the height of the curve is accompanied by a decrease in its length.

In bakehouse parlance a dough is said to ripen as fermentation proceeds, this resulting in improved bread quality. This ripening is undoubtedly the result of the changes in dough characteristics resulting from the chemical and physical processes accompanying fermentation and which are reflected in a progressive fall in the extensibility of the dough.

Fisher and Halton (3) in studying the changes in bread quality resulting from fermentation showed that the relationship between the yeast content of a dough and the fermentation time required to give optimum quality bread was logarithmic. Their curves illustrating this relationship are given in Fig. 10. A similar relationship holds between the yeast content of a dough and the time required for the extensibility to fall to any given value. Curves illustrating this are given in Fig. 11. These are based on data obtained from extensom-

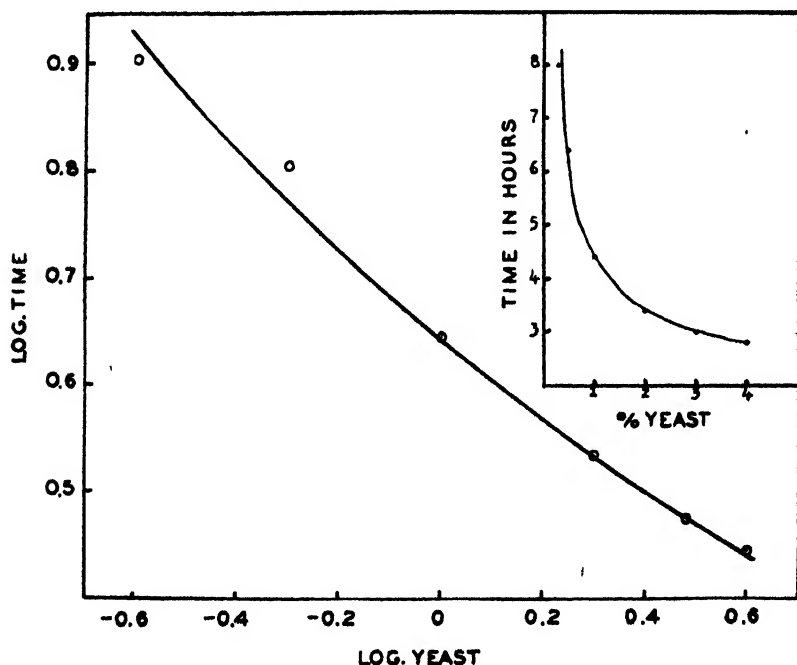


Fig. 11. Relation between yeast content of dough and fermentation time for constant extensibility.

eter curves made on doughs fermented to different lengths of time. For each yeast content the fermentation time at which the extensibility reached a value of 16 was used in constructing the curves.

The fact that the lengths of fermentation necessary to give optimum quality bread, on the one hand, and a given value of dough extensibility, on the other, are in similar relationship to the yeast content of the dough suggests that the ripening of the dough is related to the decrease in extensibility. As explained above, this decrease in extensibility is accompanied by an increase in the work hardening of the dough when stretched. This increased work hardening raises the

TABLE IV
WORK HARDENING IN TERMS OF CHANGE IN VISCOSITY
AND RIGIDITY MODULUS

	Flour 1	Flour 2	Flour 3
	<i>C.G.S. units</i>	<i>C.G.S. units</i>	<i>C.G.S. units</i>
Viscosity after 1st stretch	1.6×10^6	1.3×10^6	0.8×10^6
Viscosity after 2nd stretch	2.6×10^6	2.5×10^6	4.8×10^6
Viscosity after 3rd stretch	3.8×10^6	4.4×10^6	11.0×10^6
Modulus after 1st stretch	0.7×10^4	0.8×10^4	1.2×10^4
Modulus after 2nd stretch	0.7×10^4	0.8×10^4	1.2×10^4
Modulus after 3rd stretch	0.7×10^4	1.0×10^4	1.9×10^4

viscosity and also the relaxation time of the dough.⁴ In other words, the correctly fermented dough responds well to the stretching action of molding and, in addition, softens more slowly after molding, i.e., has a longer relaxation time. The improvement in bread quality, up to an optimum, which parallels the increased fermentation of a dough is thus a reflection of the increase in the relaxation time of the dough.

Improvers. The effects of three chemical improvers, potassium bromate, nitrogen trichloride, and ammonium persulfate, on fermented doughs is shown by the data obtained from their extensometer curves

TABLE V
EFFECT OF ADDITION OF IMPROVERS TO FLOUR
ON EXTENSOMETER CURVES

	Height	Length	Product
	<i>cm.</i>	<i>cm.</i>	
Flour A + 0 p.p.m. potassium bromate	2.0	19	38
Flour A + 10 p.p.m. potassium bromate	2.4	16	38
Flour A + 20 p.p.m. potassium bromate	2.8	14	39
Flour A + 30 p.p.m. potassium bromate	3.2	12	38
Flour B + 0 g. nitrogen trichloride per 280 lb. flour	2.5	16	40
Flour B + 3 g. nitrogen trichloride per 280 lb. flour	2.7	14.5	39
Flour B + 6 g. nitrogen trichloride per 280 lb. flour	2.9	13.5	39
Flour B + 9 g. nitrogen trichloride per 280 lb. flour	3.1	12.5	39
Flour C + 0 oz. ammonium persulfate per 280 lb. flour	2.0	22	44
Flour C + $\frac{1}{2}$ oz. ammonium persulfate per 280 lb. flour	2.4	17.5	42
Flour C + 1 oz. ammonium persulfate per 280 lb. flour	3.3	13	43

and given in Table V. The general effect is the same as that which results from fermentation; the dough becomes more elastic and less extensible, the product figure remaining unaltered. Within limits,

⁴ This is illustrated by the data given in Table IV. These data were obtained by stretching cylinders of dough on the mercury bath apparatus. Each test piece was stretched and then relaxed, the viscosity and rigidity modulus being calculated from the changes in length of the sample. This was repeated twice. The data show that the work hardening of the dough was due to an increase in its viscosity, any change in the modulus being small by comparison. Since the viscosity increased to a much greater extent than the modulus, the relaxation time also increased.

fermentation and improvers treatment are complementary, a dough having a short fermentation needing a high treatment and one having a long fermentation, a low treatment. (An unyeasted dough requires a very heavy treatment to reduce its extensibility to the value of a fermented dough.)

When a flour has been overtreated either with chemicals or by heat, or has been overfermented, the product figure falls and this is accompanied by a deterioration in bread quality.

Molding. When a dough has been correctly fermented and treated with an improver, it is brought to the condition when it shows optimum response to the beneficial effects of molding. The importance of molding is illustrated by the difference in the loaves shown in Fig. 12.

Fig. 12. Effect of molding on loaf quality.

These were made from the same flour and the doughs had identical water and yeast contents, were given the same length of fermentation, but differed in the severity with which they were molded, flour No. 1 being well molded, No. 2 more lightly handled, and No. 3 handled as little as possible.

The beneficial action of molding depends on the stretching of the dough which, by work hardening, increases its elasticity, therefore giving a more rigid structure. The efficiency of the molding process, whether given by hand or by machine, is thus an important factor in determining either bread quality or the shape of the load-extension curves. This must be considered when correlating the results of baking tests with physical tests made on the dough.

Flour Strength. Correct fermentation, improver treatment, and mechanical manipulation all condition the dough, enabling optimum quality bread to be made from any one flour. These processes bring out the potential capabilities of the flour, but they do not alter its fundamental strength.

To appreciate the meaning of flour strength in terms of the physical properties of the dough, further consideration must be given to the

experiments described earlier in this paper in which load-extension curves were obtained for cylinders of dough stretched on the mercury bath instrument.

These load-extension curves were translated into stress-strain curves using the following calculations.

If when the dough cylinder of original length l and radius of cross section r had been stretched to length l_1 , the load meter reading was Δ , then:

1. the load in dynes was $981\Delta a$ where a = force in grams necessary to cause unit deflection on the meter;
2. the cross section of the dough cylinder was $\pi r^2 \frac{l}{l_1}$;
3. the shearing stress per unit cross section was therefore:

$$\frac{981\Delta a}{3\pi r^2 \frac{l}{l_1}} = \frac{981\Delta a \times l_1}{3 \times \pi r^2 l} = \frac{\text{Load} \times \text{length}}{3 \times \text{volume}}.$$

4. the strain was $\int_1^h \frac{dl}{l} = 2.3 \log_{10} \frac{l_1}{l}.$

By plotting the calculated values of stress against strain, curves such as is shown in Fig. 13 were obtained. It will be seen that the stress on the dough increased in value until the dough rapidly tore and then broke. From the calculations given above the value of the maximum stress is given by the value of $\frac{\text{load} \times \text{length}}{3 \times \text{volume}}$ at the time when the dough ruptured. Thus for similar-sized test pieces of dough, variations on the maximum stress figures run parallel with variations in the load \times length product figures.

Experience with the extensometer has shown that the stronger the flour the greater is this product figure. It would follow, therefore, that the maximum value of the stress built up during the stretching of the dough is a measure of the strength of the flour. In other words, the strength of a flour is determined by the tensile strength of the dough.

Earlier in this paper, data, obtained from extensometer curves, were given for doughs containing increasing amounts of improvers. Attention was given to the fact that the product figure was not changed by improver addition. The significance of this will now be apparent. The fact that the improver did not change the product suggests that these chemicals have no effect on the fundamental strength of the flour. This applies to quantities of improver that are beneficial to baking quality. Overtreatment lowers the tensile strength of the dough and therefore the product figure. The results of tests described earlier in this paper showed that the product figure, for both unyeasted and

yeasted doughs, decreased in value as the doughs aged. Evidence was presented to show that this was due to a virtual increase in the water content of the dough, a change which resulted in its becoming softer and having a lower extrusion time. When the water contents of doughs, made from the same flour, were adjusted so that each, when

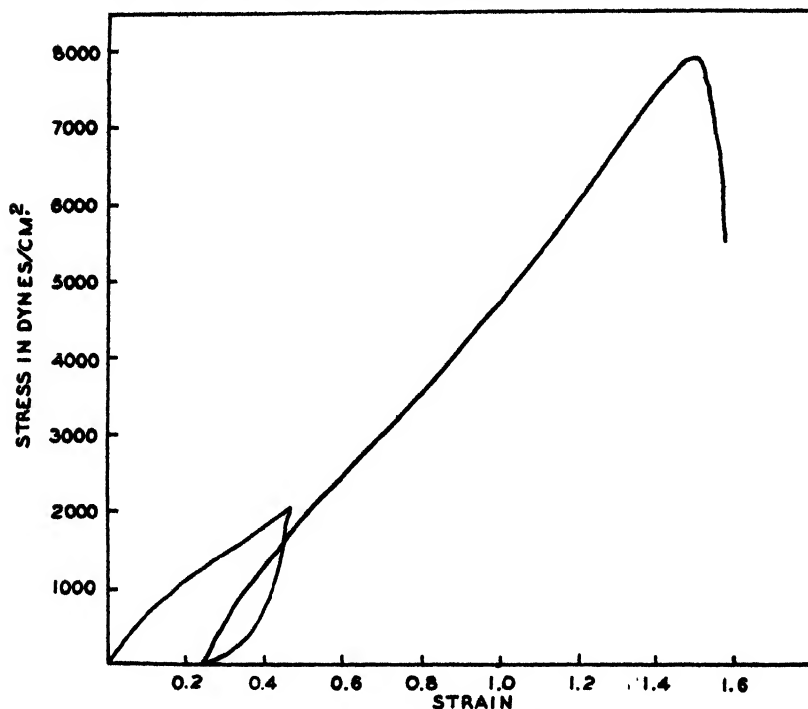


Fig. 13. Stress-strain curve calculated from load-extension.

tested, had the same extrusion time, it was found that the product figure was constant and independent of fermentation time. The results of these experiments indicate, therefore, that fermentation does not alter the strength of a flour.

The slope of the stress-strain curve is determined by the load built up during stretching. It is thus proportional to the height of the extensometer curve and is a measure of the relaxation time of the dough. Fermentation and oxidation increased the slope while reduction decreased it.

The stress-strain curve shown in Fig. 13 bends upwards slightly. With strong flours this upward bend may be quite marked, particularly when the dough is well fermented or treated with improvers. With some weak flours a downwards bend may occur. The corresponding extensometer curves are shown in Fig. 14. The increase in load with increasing extension suggests an abnormal degree of work hardening

which increases the tensile strength of the dough. The opposite effect may be due to diminishing work hardening or to the effect of structural viscosity, i.e., the fall in viscosity with increasing stress. The result is a lowering of the tensile stress.

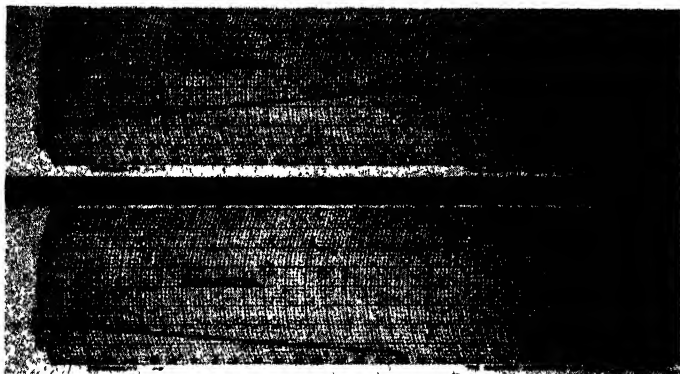


Fig. 14. Effect of strength and weakness of dough on shape of extensometer curve.

It will be realized that for any given slope of the stress-strain curve, i.e., for any given relaxation time, the greater the maximum stress the greater will be the maximum strain. In other words, the greater the tensile strength of the dough the greater will be the extensibility for a given relaxation time. Thus when properly conditioned for baking, i.e., by fermentation and improver treatment, doughs made from strong flours will stretch to a greater extent before tearing than those made from weak flours and thus should yield bread of greater volume.

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EFFECT ON DOGS OF FLOURS TREATED WITH VARIOUS IMPROVING AGENTS ¹

AARON ARNOLD ²

ABSTRACT

Dogs were fed diets with high levels of flours treated with the following agents: ammonium persulfate (15 g./cwt.), potassium bromate (5 g./cwt.), chlorine (20 g./cwt.), benzoyl peroxide (0.8 g./cwt.), nitrosyl chloride (80 g./cwt.), chlorine dioxide (5 g./cwt.), and nitrogen trichloride (5 g./cwt.). The treatments given the flours were above those used commercially so as to increase the severity of the test conditions.

In cross-over type tests, running fits were observed only in the dogs fed nitrogen trichloride treated flour. No ill effects were seen in the dogs fed untreated flour or flours treated with the agents, other than nitrogen trichloride, given above used alone or in combination.

As judged from the tests with these dogs, agents other than nitrogen trichloride appear to be acceptable for the treatment of flour intended for human consumption.

Mellanby (3) has reported recently that dogs develop running fits when fed flour treated with nitrogen trichloride, heretofore used widely for the rapid chemical maturing of flour. Dogs appear to be somewhat more sensitive to flour so treated than other experimental animals, though untoward manifestations have been produced in ferrets (4), cats (6, 7, 8), and rabbits (7). The results in rats and monkeys appear to be equivocal (6, 7, 8), while those in guinea pigs (7) and humans (6) are negative at this writing.

These findings render the continued use of nitrogen trichloride for the maturing of flour objectionable and direct attention to alternative compounds which may be used for this purpose. Accordingly, flours treated with other agents, alone and in combination, have been fed to dogs to determine whether flour so treated would cause any untoward symptoms.

Experimental

Flour Treatments. Several fairly large experimental batches of flour were prepared in the course of the study, but due to the fact that the program extended over approximately a 2-year period, it was not feasible to apply all the various agents to the same lot of flour. Further, the length of the experiment was not anticipated fully so that each agent had to be used on more than one lot of flour. In general, however, all dogs received clear grade flour during the first 3 months of the experimental feeding period and standard patent flour during the rest

¹ Manuscript received September 30, 1948.

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of the experiment. The chemical agents and the levels of treatment used, together with an approximate indication of the relation of the treatment to commercial usage, are given in the first three columns of Table I.

TABLE I
TREATMENT OF FLOUR SAMPLES USED AND SUMMARY OF DOG FEEDING TRIALS

Chemical agent	Treatment level	Approximate relation to commercial treatment level	Time fed to dogs	No. of trials	No. of trials terminated by running fits
	<i>g. per cul.</i> (45 kg.)	<i>x coml. level</i>	<i>days</i>		
Nitrogen trichloride ¹	5	2½-5	3-56	35	35 ⁶
Ammonium persulfate ^{2,3}	15	10	14-90	14	None
Ammonium persulfate ³	15				
Chlorine	20	10	26-42	4	None
Potassium bromate	5				
Ammonium persulfate ³	15				
Chlorine	20	10	21-38	10	None
Potassium bromate	5				
Benzoyl peroxide ⁴	0.8				
Nitrosyl chloride ³	80	10	23-38	10	None
Chlorine dioxide ¹	5	2½-5	16-28	5	None
None	—	—	8-14	4	None

¹ These samples were generously supplied by a nearby mill.

² In combination with inert diluents.

³ These treated flour samples were prepared in the laboratory. The nitrosyl chloride for these studies was prepared from nitric and hydrochloric acids. It was accompanied by some chlorine.

⁴ Supplied by Oxylite, available from Winthrop-Stearns, Rensselaer, New York.

⁶ Average time to develop the first observed symptoms was 12 days with a range of 3 to 56 days.

It will be noted that the treatment in each case was substantially above the level ordinarily used commercially. The high level of treatment along with the high content of flour in the diets of the dogs (approximately 80%) was chosen so that the agents studied here would be subjected to a severe test.

Dogs. Young dogs were obtained for these studies. On receipt, they were dewormed and treated with distemper vaccine. They were placed on experiment when they were fully recovered from these treatments.

Diets. To test the treated flours the dogs were fed diets patterned after those of Mellanby (3). Six days a week, each dog was offered 100-200 g. of flour (steamed 90 minutes before feeding) to which was added yeast ⁵ (5% of weight of flour), 20 g. skim milk powder, 10 ml.

⁵ Dried debittered brewers' yeast, Red Label. Vitamin Food Company, Newark, New Jersey.

corn oil, 2 g. sodium chloride, 2 g. liver concentrate powder, and 15 g. lean meat. Fish liver oil, 850 U.S.P. units of vitamin A and 100 U.S.P. units of vitamin D per ml., was given once weekly, 5 ml. per dog. In addition, each dog was given an oral supplement of 1 mg. thiamine hydrochloride, 1 mg. pyridoxine hydrochloride, 2 mg. riboflavin, 10 mg. niacinamide, 10 mg. calcium pantothenate, and 100 mg. choline chloride once weekly. The dogs had free access to drinking water at all times.

Results

The results of the dog feeding trials are shown graphically in Fig. 1. Without exception, the dogs exhibited symptoms of running fits when fed the diets which contained the nitrogen trichloride treated flour.

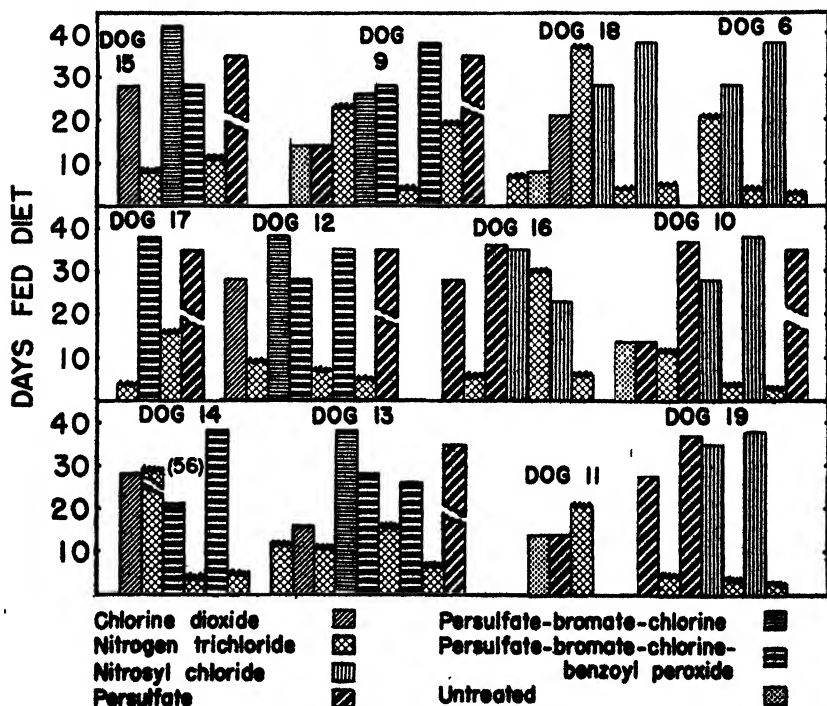


Fig. 1. Schematic representation indicating the number of days each dog was fed each diet before transfer to another diet. The development of running fits is indicated by the columns terminating in a jagged line. The interrupted persulfate columns indicate a 90-day feeding trial.

The time required for this varied and the heights of the bars show how long it took for the condition to develop to some degree in the dogs. Several factors probably operated to affect the rate of development of running fits. Some dogs reacted to being fed nitrogen trichloride treated flour by eating less; in general, these dogs developed running

fits more slowly, going for prolonged periods without symptoms, as in the cases of dogs 14, 16, and 18 (Fig. 1). The cumulative nature of the toxic principle, however, eventually precipitated the symptoms even in dogs which appeared less sensitive at times. It should be stated that, as far as possible, the dogs were transferred from the toxic diets at the earliest positive sign of the condition, for it is difficult to save a dog after a severe and prolonged attack. Some dogs were lost in spite of the attempts to prevent the development of severe symptoms.

The symptoms observed coincided with the classical description of running fits; they appeared identical with those seen earlier (1) in dogs fed a heat-processed dog biscuit of wheat flour and meat scraps. Two veterinarians associated with these laboratories concurred in the diagnosis of running fits.

No untoward symptoms were observed in the dogs fed diets which contained flour treated with other agents. The relatively high levels of treatment of the flours ($2\frac{1}{2}$ to 10, or more, times the commercial level) had no deleterious effect. The dogs fed the untreated flour remained equally symptom-free.

It will be noted also that flours treated with other agents not only failed to cause symptoms but were curative for dogs showing running fits as the result of ingesting nitrogen trichloride treated flour. It will be seen from Fig. 1 that on 28 occasions dogs with symptoms of running fits were transferred to other diets from the toxic diets. Thus the dogs recovered in nine instances when fed persulfate treated flour, in four instances when fed persulfate-bromate-chlorine treated flour, in six instances when fed persulfate-bromate-chlorine-benzoyl peroxide treated flour, in seven instances when fed nitrosyl chloride treated flour, in one instance when fed chlorine dioxide treated flour, and finally in one instance when fed untreated flour. That this was not due to the development of an insensitivity to the toxic agent is shown by the development of running fits when the dogs again were transferred to the toxic diet. Six of the dogs (dogs 9, 10, 12, 13, 15, 17) were fed persulfate treated flour for 90 days without exhibiting any untoward effects. These dogs were continued on test, along with five previously untreated animals, on diets which contained flour with 68 or 454 g. persulfate per 100 lbs. The results at the higher levels will be reported on at a later time.

The results are summarized in Table I. The summary results include one dog, not shown in Fig. 1, which was lost after 13 days on the toxic diet. The data on the weight records and food intakes of the dogs are not included. In spite of the high flour contents of the diets the dogs grew and gained weight steadily when fed the nontoxic flours.

Discussion

The foregoing results confirm Mellanby's findings (3) that feeding dogs flour which had been treated with nitrogen trichloride causes them to develop running fits. These findings are in accord with the earlier reports of Melnick and Cowgill (5), and of Arnold and Elvehjem (1), which indicated the nutritional origin of the running fits syndrome. This conclusion does not exclude the possibility, however, that a similar symptom complex may have other causes. For example, Weast, Groody, and Morgan (9) observed running fits in two dogs fed diets which contained heat treated protein (casein).

Other chemical agents that have been used in the past for the bleaching and/or maturing of flour do not appear to have similar undesirable effects in dogs. The results submitted herein may be compared with those of other investigators. Thus, flour treated with relatively large amounts of ammonium persulfate (approximately 10 times the commercial level) had no untoward effects in dogs. This is in agreement with the preliminary statement of Bentley *et al.* (2).

Furthermore, the treatment of flour by a combination of agents, ammonium persulfate, chlorine, potassium bromate, with and without benzoyl peroxide, appeared to have no unfavorable effects as judged by feeding trials with dogs. That benzoyl peroxide and chlorine tested separately have no adverse effects had been reported earlier by Newell *et al.* (6) and by Radomski *et al.* (7). Similarly, Bentley *et al.* (2) state that chlorine and potassium bromate tested separately did not produce the running fits factor in flour. Radomski *et al.* (7) also observed no untoward effects in dogs fed bromate treated flour.

Our observations that chlorine dioxide treated flours have no untoward effects when fed to dogs are in agreement with those of others (2, 6, 7).

Nitrosyl chloride, previously not reported on, also appears to produce in flour nothing having unfavorable effects in dogs.

Thus nitrogen chloride alone, of all the agents tested thus far, produces a factor in flour which, fed to dogs, causes the rapidly and progressively fatal running fits syndrome:

Acknowledgments

The writer is indebted to Dr. O. H. Siegmund and Dr. E. J. Morrison of these laboratories for examination of some of the dogs exhibiting running fits. The writer also wishes to express his appreciation to John C. Eggers and George M. Harder for their help in the care of the animals.

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HEAT OF HYDRATION OF CERTAIN WHEAT FLOURS AND GLUTEN ¹

W. G. SCHRENK,² A. C. ANDREWS, and H. H. KING

ABSTRACT

The heats of hydration of moisture-free samples of wheat flour and of gluten have been measured calorimetrically by the use of a Dewar type calorimeter, a thermocouple, and a potentiometer. Wheat flour has a heat of hydration of approximately 27 calories per gram. Slight variations occur between different flours, but they do not seem to be correlated with other properties of the flour. The rate of hydration of flour or gluten is increased by the addition of a wetting agent, without materially altering the magnitude of the energy released.

The heat of hydration of gluten (approximately 20 calories per gram) is less than that for flour, and the reaction proceeds much more slowly. This rate can also be increased by the addition of a wetting agent. The addition of cysteine to the water solution does not affect the rate or the magnitude of the energy of hydration of gluten.

Specific heat values obtained on flours and starches range from 0.42 to 0.48 calories per gram.

Winkler and Geddes (11) have shown that heats of hydration of flour and of starches vary slightly with the source of material. They made a number of measurements of this property on these materials, as well as of the specific heat of these substances. Daniels, Kepner, and Murdick (1) also made a few determinations of heats of hydration

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² Portion of a thesis presented as partial fulfillment of the requirements for the degree Doctor of Philosophy in chemistry at Kansas State College.

and of the specific heat of flour. The papers agree well on specific heat data and both agree as to the magnitude of the heat of hydration. Winkler and Geddes (11), however, indicate that the magnitude of the heat of hydration is greatly influenced by the residual moisture content of the sample, but they made no attempt to obtain a sample which was entirely free of moisture; rather, they determined the heat of hydration at various low moisture levels. Their results under these conditions indicate differences in the heats of hydration of starches and of flours from different sources.

Since the data contained in the papers mentioned do not give maximum values for the heat of wetting because of the moisture present, and were obtained by the use of relatively large concentrations of material, it seemed desirable to attempt an accurate measurement of heats of hydration at minimum moisture levels and in dilute mixtures of the samples and water, and thus attempt to obtain a measurement of maximum value for the energy of hydration. These data would then be available as fundamental information and for use in possible correlations with other known properties of the materials.

The energy of hydration of gluten was also considered important from the standpoint of total energy of hydration of flour, and data on several samples of gluten are included. A few specific heat measurements were also made in order to verify existing data. The effect of the presence of a wetting agent was also determined since it is known that wetting agents have an effect on mixogram patterns (7) and consequently affect baking qualities.

Materials and Methods

Preliminary experiments indicated that the imbibition of water by flour or gluten was quite rapid, and that the use of a Dewar flask type of calorimeter was adequate for the measurements of heats of hydration. The arrangement of the calorimeter and associated equipment has been previously described (6).

Temperature measurements were made by the use of a calibrated 50 junction copper-constantan thermocouple and a type K potentiometer. With the galvanometer arrangement used a change in potential of 0.001 mv. could be detected. This potential difference, together with the sensitivity of the thermocouple, gave a sensitivity of $\pm 0.00046^\circ\text{C}$.

Determination of the Heat of Hydration. The technique of making measurements of the energy of hydration was similar to that developed for the heats of immersion of paint pigments by Harkins and Dahlstrom (2). The method was essentially as follows: A sealed sample tube containing the sample was placed in the calorimeter immediately

above 400 to 425 g. of distilled water. The sample tube was arranged so that the bottom could be broken from outside the calorimeter and the sample allowed to fall into the water. All the contents of the calorimeter were brought to the temperature of the thermostat in which the calorimeter was immersed. After temperature equilibrium had been attained the stirrer was started and electrical connections made.

Temperature readings were then taken at one-minute intervals in order to establish the slope of the time-temperature curve. The sample was then introduced into the water by breaking the sample tube. Readings were continued and the energy released by the reaction was measured by means of the temperature rise produced. After the temperature rise had reached its maximum, readings were continued in order to re-establish the slope of the time-temperature curve. This was followed by the addition of electrical energy to the calorimeter by means of a current passing through the heater coil in the calorimeter for a known period of time. Current was measured with a potentiometer and a standard one-ohm resistance. Current drain was low and the source of current was heavy-duty storage cells. A small vernier resistance permitted precise control of current. The temperature rise produced by the electrical energy was measured as before.

Fig. 1 represents the type of curve obtained by such measurements. The solid line represents the results to be expected if the energy releases and temperature rises were instantaneous. The segment *AB* represents the establishment of the time-temperature curve previous to breaking the sample tube. *BC* represents the rise in temperature produced by the release of the energy of hydration; *CE* represents the new time-temperature slope; and *EF* represents the rise in temperature produced by the electrical energy released by the heater coil.

The electrical energy released, and represented by *EF*, can be calculated in calories as follows:

$Q_2 = \frac{I^2 R t}{4.1833}$ where *I* is the current in amperes, *R* is the resistance of the heater coil in ohms, and *t* is the time in seconds.

If *Q*₁ represents the energy required to produce a temperature rise equal to *BC*, it follows that

$Q_1 = Q_2 \frac{BC}{EF}$ where *Q*₁ is the energy of hydration of the sample.

The total rise in temperature during the course of an experiment was approximately 0.8°C. A typical set of data is shown in Fig. 2.

This method of making measurements eliminates a number of troublesome calculations, including energy equivalence of the calori-

meter, specific heat of the sample, and accurately measured amounts of water. Temperature measurements do not need to be changed to degrees, but may more conveniently be expressed in terms of millivolts. The accuracy of measurement thus depends on the accuracy of the measurements of the electrical constants and the precision of timing.

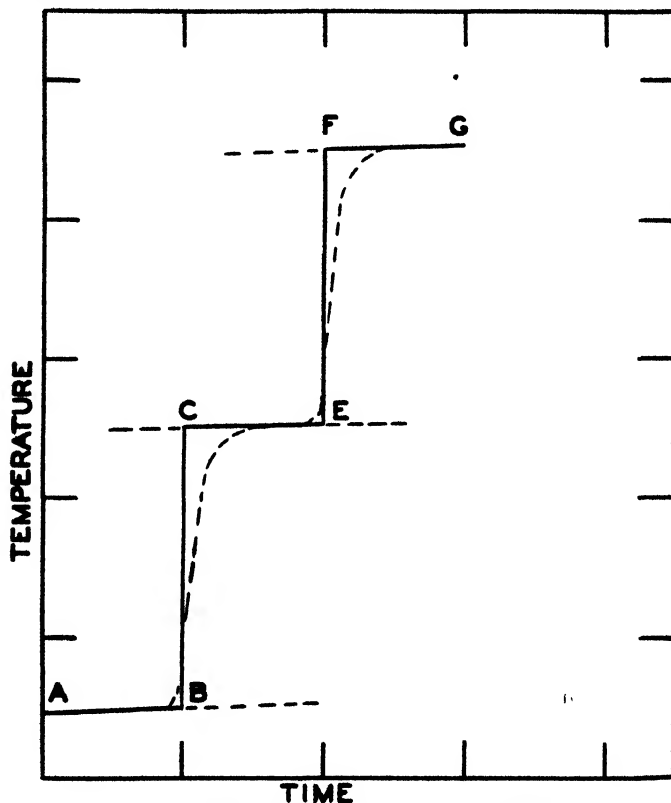


Fig. 1. Method of calculating the temperature rise.

The calorimeter and measuring equipment was checked for accuracy against the heat of neutralization of hydrochloric acid with sodium hydroxide. Excellent agreement with the data of Richards and Rowe (4) was obtained.

Preparation of Samples. Samples were prepared by drying overnight in a vacuum oven at 100°C . They were then placed in sample tubes as previously described (6) and dried under vacuum again. The tubes were sealed under vacuum. Numerous moisture determinations made on samples prepared in this way indicated that essentially no moisture remained. The tubes were placed in the calorimeter and allowed to reach temperature equilibrium with the contents after

which they were broken in such a way as to get rapid mixing of the sample and water (6). The entire calorimeter was immersed in a constant temperature water bath maintained at $25 \pm 0.1^\circ\text{C}$., and all measurements were made at this temperature.

The literature on the effect of the heat treatment used in drying these samples is not entirely in agreement. Winkler and Geddes (11)

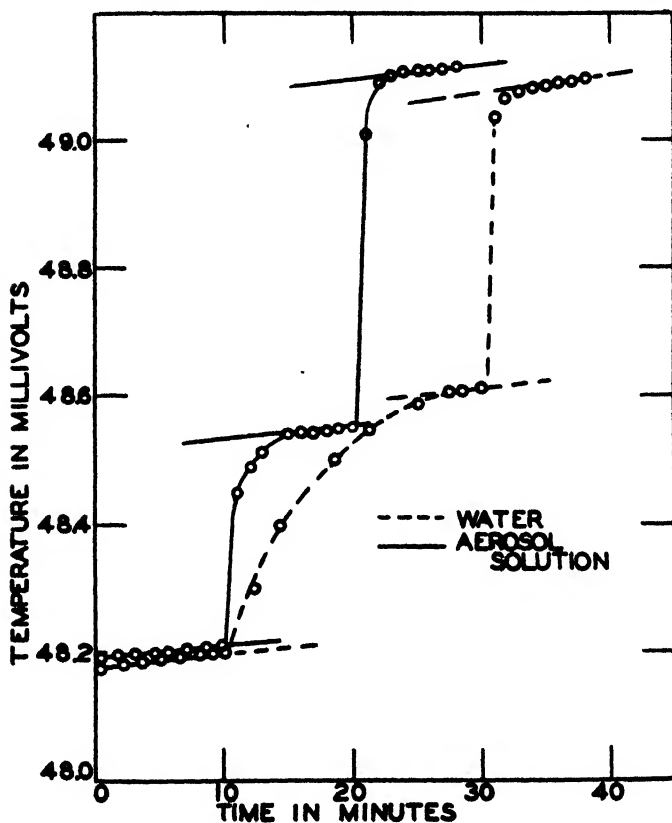


Fig. 2. Effect of aerosol OT on the time-temperature curve of gluten.

have shown that heat treatment affects the hydration reaction slightly. Sair and Fetzer (5) also reported that the sorption capacity of starch is somewhat altered by heating. Hellman and Melvin (3) indicated that in a series of corn starches from different sources and treatments, there was no significant change in moisture sorption capacity. Wands (10) also has recently reported on the sorption of water by starches, in which some samples were autoclaved at high temperature and pressure. This treatment produced no measurable change in the water sorption capacity of the starch. In view of the nature of the above data it

seems likely that the heat treatment accorded these samples produced relatively little effect. Furthermore, since all samples were accorded the same treatment, any slight changes produced would be similar and comparisons would remain valid. It is very likely that any remaining traces of moisture would have a much greater influence on the data than did the heat treatment.

Determination of Specific Heat. Specific heat calculations require precise data on the energy equivalence of the calorimeter. This was determined by placing a known quantity of water in the calorimeter, adding a known amount of electrical energy, and calculating the temperature rise. Specific heat measurements were then made in the same manner with the sample in place. The sample was packed tightly into a long, thin-walled brass tube which was then immersed in a known quantity of water in the calorimeter. Time was allowed to permit the calorimeter and its contents to reach temperature equilibrium at 25°C. Electrical energy was then added as before and the rise in temperature calculated. From these data the specific heat of the sample could be calculated since all other factors were known.

Results and Discussion

Heat of Hydration of Flour. The heats of hydration determined on a number of different samples of wheat flour vary over only a narrow range (Table I), except for sample 3 which gave a very high value. This discrepancy may be due to the fact that this sample had been bleached with chlorine, the only flour in the series which was so treated.

The time required for the hydration reaction to reach completion was also quite constant. An average time of about 9 minutes was required. This is considerably longer than for starch alone, which averaged about 4 minutes. Gluten, in contrast, required about 17 minutes. Evidently the reaction time for flour is influenced considerably by the presence of the gluten in the flour.

The data in columns 2, 3, and 4 of Table I were obtained from measurements made with the use of the recording dough mixer (9). These properties do not present any evident correlation with the heat of hydration values. Tenmarq and Chiefkan flour, which are quite different in baking properties, also do not show measurable differences in their heats of hydration.

Hydration of Gluten. The data in Table II present information on the energy of hydration of gluten. Gluten releases its energy of hydration much more slowly than starch or flour. Grinding of the gluten made no difference in the time required for hydration. The energy of hydration of the gluten is considerably below that of flour and starch, averaging about 20 calories per gram.

TABLE I
COMPARISON OF THE HEAT OF HYDRATION OF FLOURS WITH CERTAIN
OTHER PROPERTIES OF FLOURS AND THEIR DOUGHS¹

Sample	Nature of flour	Protein ²	Mixogram curve measurements			
			Mixogram angle at peak	Area under curve	Height	Heat of hydration
		%	degrees	cm. ²	cm.	cal./g.
1	Cake	9.4	6	33.7	2.2	27.6
2	Cake	8.8	4	39.4	2.9	27.2
3	Cake (Cl ₂ bleach)	12.0	19	72.8	6.2	30.0
4	Bakery	11.7	25	71.4	6.0	27.2
5	Bakery	12.9	12	79.4	5.8	26.8
6	Cracker dough	9.4	7	38.1	2.8	27.3
7	Cracker dough	9.5	3	42.9	3.0	26.4
8	Cracker sponge	10.8	25	61.4	5.6	27.5
9	Cracker sponge	10.9	10	73.0	5.0	25.2
10	Family	11.6	10	74.1	5.4	26.8
11	Family	10.2	14	64.0	5.7	26.2
12	Cooky flour	8.1	2	37.2	2.2	26.0
13	Cooky flour	10.8	7	63.7	4.7	27.2
14	White bread	14.6	13	90.1	6.3	26.4
15	Topping	12.1	7	70.4	4.9	28.2
16	Tenmarq flour	—	—	—	—	27.0
17	Chiefkan flour	—	—	—	—	27.1

¹ All samples dried as explained in the experimental section.

² At zero moisture content.

TABLE II
HEAT OF HYDRATION OF GLUTENS IN WATER AND WATER SOLUTIONS¹

Source of gluten	Treatment	Time for hydration	Heat of hydration
		min.	cal./g.
Chiefkan wheat	None	18	20.0
Tenmarq wheat	None	18	20.2
Chiefkan wheat	1% Aerosol	6	19.8
Tenmarq wheat	1% Aerosol	6	19.5
Chiefkan wheat	0.5 g. Cysteine	19	19.8
Tenmarq wheat	0.5 g. Cysteine	18	21.0

¹ All samples dried as explained in the text.

Effect of Adding a Wetting Agent. Swanson and Andrews (7) have shown that the addition of a wetting agent markedly changes the shape of the mixogram patterns in flour and consequently affects the baking qualities of the flour. The addition of a wetting agent (1% Aerosol OT²) reduced the time required for the release of the energy of hydration of gluten to about 6 minutes.

² Obtained from American Cyanamid and Chemical Corporation.

This change in time is illustrated by the two curves of Fig. 2. The vertical heights of the lower segments of the two curves are not comparable because of different sample weights.

The magnitude of the energy released was not greatly changed. Apparently the reduction in interfacial tension produced by the addition of the wetting agent permitted the water to penetrate into the particle much more rapidly and thus allowed more rapid release of the energy of hydration without seriously affecting its magnitude. This more rapid penetration would have a marked effect on the mixogram pattern of flour regardless of the energy changes involved. An increased reaction rate for flour was also observed when the wetting agent was used for these materials.

Effect of Adding Cysteine to Gluten. Swanson and Andrews (8) have shown that the addition of cysteine to a dough produces a marked effect on mixogram patterns. The effect of cysteine on mixogram patterns is not reflected in measurements of the heat of hydration since it did not change the heat of hydration of gluten or the rate of release of energy.

Data on the effects of cysteine and aerosol OT are presented in Table II.

Specific Heat of Flour and Starch. The specific heat of flour and starch has been previously determined by Daniels *et al.* (1) and also by Winkler and Geddes (11). Since the calorimetric equipment used here offered an excellent opportunity to check these data, several experiments were performed to determine this property of flour and starch. The data obtained on several samples are given in Table III.

TABLE III
SPECIFIC HEAT OF SEVERAL STARCHES AND FLOURS

Sample	Specific heat
	Cal./g.
Wheat starch	0.48
Wheat starch	0.47
Wheat flour	0.45
Wheat flour	0.43
Waxy corn starch	0.46
Waxy corn starch	0.44
Blackhull starch	0.45
(from sorghum grain)	
Blackhull starch	0.42
(from sorghum grain)	

These data may be compared with the value of 0.397 reported by Winkler and Geddes (11), and 0.425 reported by Daniels *et al.* (1). The specific heat of the samples of wheat starch is slightly greater than that of wheat flour, waxy corn starch, and sorghum starch.

Acknowledgment

Appreciation is expressed to the Department of Milling Industry of Kansas State College and especially to Dr. J. A. Shellenberger of that department for supplying the samples used; also, for permission to use the data obtained with the recording dough mixer presented in Table I.

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THE BIOLOGICAL VALUE OF SOME COMMERCIAL CORN PROTEIN FRACTIONS¹

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ABSTRACT

A number of workers have emphasized recently the potential value of corn germ in the human dietary. With few exceptions the corn germ investigated to date has been produced by the dry milling of whole corn. In this study, the materials used were commercial by-products of the wet-milling of whole yellow corn. They were steep water concentrate, corn gluten, and variously processed corn germ.

A series of nitrogen metabolism trials were conducted, using groups of mature male rats, to determine the effects of method of processing and aging on the coefficient of true digestibility and biological value of certain of these nitrogen supplements.

The protein of corn germ that had been expeller processed was less efficiently utilized biologically than the same corn germ which had been solvent-extracted to remove the oil.

Solvent-extracted corn germ that had been aged at room temperature a few months, two years, and six years yielded biological values of 83 ± 1.5 , 79 ± 1.9 , and 55 ± 1.4 , respectively, demonstrating unmistakably the deleterious effect of storage.

The feasibility of conducting the same rat through three nitrogen balance tests is discussed.

Recently, a number of authors have reported upon the protein value of corn germ, emphasizing its potential value in the human dietary. The nutritional limitations of corn and certain of its by-products were demonstrated years ago by Osborne and Mendel (15, 16). In these early studies, growth response made by rats was the index of quality. Later Mitchell (9, 10), using a less empirical procedure, found the biological value of the proteins of whole corn to be 72 and 60 when included in the ration at levels of 5 and 10%, respectively.

Mitchell and Beadles (12), utilizing the Mitchell nitrogen balance method, found the biological value of the protein of dry-milled solvent-extracted corn germ to be 77.6, which was equal to that of round of beef and 85% as digestible. Jones and Widness (6) compared the growth-promoting values of wheat germ and corn germ proteins with those of various other vegetable and animal proteins by feeding them to weanling rats at protein levels of 10, 15, and 17.5% of the ration. At all of these levels, they found wheat germ protein to be superior to corn germ protein.

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Recently Beeson, Lehrer, and Woods (1) reported upon the protein values of wheat germ and corn germ using the growth-promoting method with young rats. As sole source of protein in the ration, both wheat germ and corn germ compared favorably with whole egg protein in the rate of gain made by the rats, but the corn germ was less efficient. As a co-supplement with peas, corn germ protein fed at various percentage levels also was somewhat less efficient than wheat germ under the conditions of their experiments.

Stare and Hegsted (17) confirmed the relative superiority of wheat germ compared to corn germ as a supplement to wheat flour, but found that for maintaining the nitrogen balance in the adult dog, the proteins of skim milk powder, wheat germ, and corn germ were practically of equal value.

Hove and Harrel (4) reported corn oil meal (corn germ) to have a protein value of 2.40, which represented grams gain in weight made by rats per gram of protein eaten. On the same basis, they found corn oil meal to have a protein value of 1.71 as a supplement to white patent flour, compared to a protein value of 2.26 for both dry skim milk and defatted wheat germ. Soybean oil meal on this basis had a protein value of 1.77. Block and Bolling (2) reported the protein value of solvent-extracted corn germ to be 1.8 while corn germ extracted by the "hot expulsion method" had a protein value of 2.1.

With few exceptions the corn germ investigated to date has been produced by the dry milling of whole corn. In this study, the by-products used were commercial products of the wet-milling of whole yellow corn. By far the largest percentage of commercial corn proteins on the market is produced by this method.

Materials and Methods

This test comprised a series of nitrogen metabolism trials in which groups of mature male rats six months old and weighing from 250 to 350 g. at the start of the experiments were used to determine the coefficient of true digestibility and biological value of various protein by-products of the corn milling industry. The supplements tested and pertinent remarks concerning their preparation are given in Table I. Brief reports concerning these have appeared in the annual reports of this station (3).

Each supplement was fed to groups of 11 rats. Uniform environmental conditions were maintained for all rats throughout the period of each test and insofar as possible between tests. Each test material was included in a diet which we believe was otherwise nutritionally complete for mature rats and was fed *ad libitum* for a period of 14 days to rats caged separately. Each rat was conditioned to its test ration

during the first 7 days of this period. A 14-day low-nitrogen period, during which the rats received only the basal diet, both preceded and followed each test nitrogen (nitrogen assay) period. Data pertinent to the determination of coefficients of these digestibility and biological values were collected during the last 7 days of each 14-day period.

TABLE I
PROTEIN SUPPLEMENTS USED AND THEIR METHOD OF PREPARATION¹

Expt. no.	Supplement	Percentage protein ²	Remarks
1	Corn steep water concentrate	27.10	Contains acid and water-soluble portions removed by steeping the corn and washing the starch.
2	Corn germ ³	20.75	Oil removed by laboratory extraction with diethyl ether.
8	Corn germ ³	20.75	Oil removed by laboratory extraction with diethyl ether, then aged for two years at room temperature (70°-90°F.) in metal container.
20	Corn germ ³	20.75	Oil removed by laboratory extraction with diethyl ether, then aged at room temperature for six years. Had acquired a musty and somewhat rancid odor.
3	Corn germ	25.30	Oil removed commercially by expeller process.
4	Corn gluten	44.00	Standard commercial product.
5	Soybean oil meal	44.46	Oil removed commercially by solvent extraction. Meal had aged several years prior to its use experimentally.

¹ All supplements except soybean oil meal are products of commercial wet-milling process. Samples utilized in Experiments 1-4 inclusive were supplied by the American Maize Products Company through the courtesy of H. H. Schopmeyer.

² Protein = $N \times 6.25$.

³ Represent same corn germ aged differently as noted.

The basal, low-protein ration had the following percentage composition: starch 86, filtered butterfat 8, McCollum's salts No. 185 (7) 4, and agar-agar or celluration (Fisher) 2%. The protein supplements to be tested replaced equal parts by weight of the starch so that the test rations contained approximately 5% crude protein. The actual percentage of protein in each ration was determined chemically. Each rat received in addition 50 mg. of yeast concentrate (Harris) and 20 μ g. thiamine chloride daily, and three drops of a cod liver oil concentrate (Upjohn) weekly.

The ration for each rat was weighed out daily. It was placed in feeding devices so constructed that spillage was practically eliminated. Uneaten feed was weighed back each morning. Whenever feed was spilled, it was added to the next day's ration so that each rat actually ingested all the feed with which it was credited. Each rat was offered daily all the feed it could consume. Distilled water was fed *ad libitum*.

Feces and urine were collected quantitatively and preserved for analysis each day during the last 7 days of the period. The urine, which was absorbed on acidulated filter paper, and the feces were pre-digested with 20% hydrochloric acid solution. At the close of the 7-day collection period, aliquots of the digests of both total urine and feces were further digested with sulfuric acid. Nitrogen was determined by the Kjeldahl method using copper sulfate as a catalyst. Except for minor modifications, the method used for determining the coefficients of true digestibility and biological value of the proteins was that developed by Mitchell and associates (9, 10, 13).

Results

The extent to which rats digested or utilized the proteins in steep-water concentrate, variously processed corn germ, and corn gluten is reported in Table II. All of these supplements were highly digestible.

TABLE II
UTILIZATION OF PROTEIN FROM DIFFERENT SUPPLEMENTS BY MATURE RATS

Supplement	Expt. no.	Rat nos.	Percentage protein ¹		Coeff. of true digestibility ²	Biol. value ³	Avg. wt. change of rats ⁴
			Supplement	Ration			
Steep water concentrate (from corn)	1	1-11 ⁵	% 27.10	% 6.19	Avg. 88±1.0	Avg. 35±2.6	g. -5.8
Corn germ (commercial) ether extracted ⁴	2	1-11	20.75	5.37	72±0.9	83±1.5	+6.0
Corn germ (commercial expeller process) ⁴	3	12-22	25.30	5.18	70±1.0	66±1.2	+1.6
Corn gluten (commercial)	4	12-22 ⁵	44.00	5.36	89±0.7	68±2.6	-9.5
Soybean oil meal (solvent extracted)	5	12-22	44.46	5.49	83±0.9	67±1.5	-5.9

¹ Protein = N × 6.25.

² Computations based on a 7-day collection period following a conditioning interval of similar length. ± = probable error.

³ Same rats used in Experiments 1 and 2, and in 3, 4, and 5.

⁴ Both samples of germ derived from same batch of whole corn.

⁵ One of 11 starting rats "off feed" during test. Calculations are mean values based on data from remaining rats.

The biological values of their proteins differed greatly. As anticipated, steep water concentrate had a biological value of only 35. On the other hand, solvent-extracted wet-milled corn germ (Experiment 2) gave a biological value of 83. This value would seem to compare favorably that with of 77.6 obtained for dry-milled solvent-extracted corn germ by Mitchell and Beadles (12), even though our corn germ was fed at a 5% level to mature rats, and theirs at a 10% level to growing rats. Stare and Hegsted (17) state that the wet-milling process probably destroys some of the nutritive value of corn germ.

If this is so, it would seem as though the magnitude of destruction is small judging from the results presented here.

Recently, Mitchell, Hamilton, and Beadles (14), while demonstrating that the biological value and digestibility of the proteins of soybeans could be improved by mild heat-processing, commented upon the sensitivity of the cereal proteins to heat and warned against some of the methods used in their preparation. The correctness of this statement seems to be borne out by the results of Experiments 2 and 3 reported in Table II. Both samples of corn germ were derived from the same batch of whole corn and both were commercially wet-milled. That used in Experiment 2 was subsequently laboratory-extracted with diethyl ether, whereas the germ used in Experiment 3 had been expeller-processed. Both samples of germ were equally digestible; however, the expeller-processed germ had a biological value of only 66 as compared to 83 for the germ solvent-extracted in the cold. The difference in these results very probably indicates the effect of heat treatment during expeller-processing upon the native proteins of the corn germ. The biological value of 68 established in Experiment 4 for corn gluten demonstrates that the proteins of this supplement were utilized as well as those of the expeller-processed corn germ (Experiment 3).

TABLE III

EFFECT OF LENGTH OF STORAGE AT ROOM TEMPERATURE OF A SAMPLE OF ETHER-EXTRACTED CORN GERM UPON THE UTILIZATION OF ITS PROTEIN BY MATURE RATS

Duration of storage	Expt. no.	Rat nos.	Percentage protein ¹		Coeff. of true digestibility ²	Biol. value ³	Avg. wt. change of rats ³
			Corn germ	Ration			
<i>Years</i>					<i>Avg.</i>	<i>Avg.</i>	<i>g.</i>
$\frac{1}{4}$	2	1-11	20.75	5.37	72 \pm 0.9	83 \pm 1.5	+6.0
2	8	23-33	20.75	5.02	81 \pm 1.0	79 \pm 1.9	+3.8
6	20	69-79 ⁴	20.75	5.09	76 \pm 0.8	55 \pm 1.4	-3.1

¹ Protein = N \times 6.25.

² Computations based on a 7-day collection period following a conditioning interval of similar length. \pm = probable error.

³ Four of 11 rats started refused to eat ration. Calculations are mean values based on data from remaining rats.

Jones, Divine, and Gersdorff (5) and Mitchell (11) have reported that the storage of either whole or ground corn for periods of several months or longer resulted in a decrease in the biological value of the proteins of the corn. The data presented in Table III show the effect of storage upon the biological value of the ether-extracted sample of commercial corn germ used originally in Experiment 2. Note that this sample showed a biological value of 83 and a coefficient of true digesti-

bility of 72 (Experiment 2). It was stored for 6 years at room temperature and biologically tested when 2 and 6 years old. The harmful effect of storage on the ether-extracted germ was moderate after 2 years, but very pronounced after 6 years. The coefficient of true digestibility had, however, risen from 72 to 81 after 2 years' storage, which is a significant increase.

The corn germ which had been stored for 6 years at room temperature had a musty, somewhat rancid odor so that at the beginning of the 14-day test period of Experiment 20, only a few of the experimental rats ate liberal amounts of the ration into which it was incorporated. However, by the time the preliminary 7-day period was completed, seven of the 11 rats were eating the diet satisfactorily. These seven rats established a biological value of 55 for the 6-year-old germ in marked contrast to 83 for the same germ comparatively unaged. The coefficient of true digestibility of 76 for the 6-year-old germ was between the value of 72 for the fresh sample and 81 for the 2-year-old sample. No explanation for these moderate shifts in the digestibility of this germ can be offered at this time; however, the effect of the lengthy storage upon the biological value of the proteins is unmistakable.

Discussion

There has been some question whether the same group of rats should be conducted through more than two protein test periods. Rats no. 12-22 after having been subjected to two 14-day protein test periods and three 14-day low-protein periods, although somewhat attenuated, established in Experiment 5 an average biological value of 67 for a sample of solvent-extracted soybean oil meal. This value compared favorably with the figure of 67.5 obtained with the rats used by Mitchell and Beadles (12). Mitchell and Beadles used young rats and fed autoclaved soybeans at a protein level of 10% of the ration. Melnick, Oser, and Weiss (8) obtained a biological value of 71 for autoclaved soybeans when fed at a protein level of approximately 9% of the ration. It would appear that the biological value of 67 (Experiment 5) is not in disagreement with the values obtained by others when it is considered that our sample of solvent-extracted soybean oil meal possibly had been heated insufficiently (8), had been stored at room temperature for several years (10), and was fed at a 5% protein level. Rats 23-33 (Table III, Experiment 8) established a biological value of 79 for corn germ stored 2 years, after satisfactorily establishing two previous biological values for as many different supplements; thus again indicating that it is feasible to conduct the same rats through three protein test periods.

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THE BIOLOGICAL VALUE OF THE PROTEINS OF CORN GERM AND ENDOSPERM WET-MILLED EXPERIMENTALLY¹

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ABSTRACT

The coefficients of true digestibility and biological value have been determined, using conventional procedures, primarily for samples of germ and endosperm produced by the experimental wet milling of certain inbred lines and a related hybrid of corn. The inbred lines tested were OS426 waxy and starchy and 1205 waxy and starchy; the hybrid, Iowa 939 waxy.

No highly significant differences were found between the biological values of the germ and endosperm proteins from the waxy and starchy inbreds, nor between these and comparable protein fractions from the hybrid Iowa 939 waxy. It is recognized that more marked differences in biological values might have been obtained had the genetic lines used been more unrelated.

None of the proteins used in this study produced the high biological values exhibited by a commercial wet-milled sample of corn germ reported in a previous paper. This may be due to the fact that the commercial nitrogenous by-products used in the previous study were derived from corn of more heterogeneous origin while the present samples were from Iowa 939 and its single-cross inbred parents. These differences may also be due to variations in the commercial and laboratory technique used in wet milling, resulting in somewhat differently constituted end products.

It has long been known that the percentage of crude protein in the corn kernel could be influenced by proper management and fertilization of the soil in which the corn was grown. Very recently, Richter (9) increased the crude protein of Iowa 939 corn from 8.8 to 9.4% by fertilization of the soil. Whether the biological values of the corn proteins were influenced by such cultural procedures is not known. It is well established that the percentage of crude protein can be influenced by the genetic constitution of the corn. Certain corn hybrids differ markedly in the percentage of crude protein they contain, and there is some evidence that the biological values of the proteins of such hybrids also may differ. However, accumulated evidence bearing on the latter is still fragmentary.

Kik (3) found that various varieties of rice differed in the biological value of their proteins. Marais and Smuts (8) reported the biological value of "whole white maize" to be 76 ± 1.91 while that of "whole yellow maize" was 67 ± 0.98 . Doty, Bergdoll, Nash, and Brunson (1) showed that "the amounts of cystine, arginine, histidine, tryptophan, and tyrosine present in two samples of single-cross hybrid corn

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² Research Assistant Professor and Research Professor Animal Chemistry and Nutrition Sub-section.

differed considerably as did the percentages of alcohol and alkali soluble nitrogen."

The high biological value of 83 obtained with a sample of commercial wet-milled corn germ in 1940 (2), and the discovery in 1942 (2) that the protein of the germ from a sample of laboratory wet-milled Iowa 939 waxy corn had a much lower biological value, namely 66, raised the question whether or not this lower figure resulted from genetic characteristics inherent in the waxy strain of Iowa 939. At that time, Iowa 939, whose starch had been found to be an acceptable substitute for tapioca starch, had just been developed. Limited amounts of corn from the inbred lines of OS426 waxy and starchy, and I205 waxy and starchy employed in the production of the hybrids Iowa 939 waxy and Iowa 939 nonwaxy became available. The coefficients of true digestibility and biological values of certain nitrogenous by-products derived from this genetic material constitute the basis of this report.

Materials and Methods

The samples of corn referred to above were experimentally wet-milled.³ Samples of diethyl ether-extracted corn germ and of the corn endosperm were made available. Also provided for study was a sample of OS426 starchy corn endosperm which had been extracted with ethanol to remove most of the zein. The coefficients of true digestibility and biological values were determined by the same procedure as outlined in a companion article (10). Briefly, each test material was fed individually to groups of 11 mature male rats. All rats were conditioned during the first 7 days of a 14-day test period. Data concerning feed eaten and urine and feces elimination were collected quantitatively during the last 7 days and appropriately preserved for chemical and statistical analysis.

The test materials were included at the 5% level in a basal ration (10) nutritionally complete for rats except for the protein. The ration for each rat was weighed out daily, and placed in feeding devices so constructed that spillage was almost completely eliminated. Any spilled or unconsumed feed was accounted for quantitatively.

Except for minor modifications, the procedure used in determining biological value and coefficient of true digestibility of the test materials was that developed by Mitchell and associates (4, 5), as specified in the preceding paper (10).

³ The samples of inbred lines of corn used in this project were furnished us by courtesy of Dr. G. F. Sprague, Senior Agronomist, U. S. D. A., Ames, Iowa. They were grown on test plots during the same season under comparable conditions and processed at the same time so that possible differences in soil fertility, available moisture, stage of maturity, storage, etc., and their effects on the quality of the proteins should not be a consideration here.

The whole corn samples were wet-milled by the Plant Chemistry Subsection of the Iowa Agricultural Experiment Station. The endosperm samples contained all the endosperm of the corn while the germ samples were as free from adhering endosperm as was possible with the experimental method of wet milling employed.

Results

Data concerning the quality of the protein and its digestibility in each of the samples of corn germ tested are reported in Table I.

TABLE I
UTILIZATION OF PROTEIN FROM CORN GERM OF DIFFERENT GENETIC
CONSTITUTION AS MEASURED BY MATURE RATS

Source of germ	Expt. no.	Rat nos.	Percentage protein ¹		Coeff. of true digestibility ²	Biol. value ³	Avg. wt. change of rats ³
			Germ	Ration			
			%	%	Avg.	Avg.	-g.
OS426 waxy ⁴	10	34-45 ⁴	25.03	5.11	83±1.1	72±1.4	+0.5
OS426 starchy	9	34-44 ⁴	25.63	4.94	82±1.0	72±1.6	+1.7
I205 waxy	11	34-45 ⁴	26.37	5.11	85±0.5	67±1.4	+0.6
I205 starchy	12	46-56	23.91	5.38	79±0.5	64±1.7	-7.4
I205 waxy	13	46-56	—	5.07	81±0.6	65±1.3	+2.7
I205 starchy							
OS426 waxy							
OS426 starchy (equal parts of each)							
Iowa 939 waxy	7	23-33	24.27	5.02	83±0.6	66±1.2	-5.4

¹ Protein = $N \times 6.25$.

² Computations based on a 7-day collection period following a conditioning interval of similar length. \pm = probable error.

³ Corn from inbred lines used in this study obtained by courtesy of G. F. Sprague, Senior Agronomist, U. S. D. A., Iowa State College, Ames, Iowa. All samples were wet-milled in the laboratories of the Plant Chemistry Subsection of the Iowa Agricultural Experiment Station.

⁴ Rat no. 35 became sick during Experiment 9 and was discarded. Replaced by no. 45 in Experiments 10 and 11.

Apparently there are no highly significant differences between the average biological values of the various waxy and starchy inbreds, nor between these and a composite of them, or hybrid Iowa 939 waxy. The largest differences in average biological values are between those of OS426 waxy or starchy and the following: the I205 inbreds; a composite of all inbreds; and Iowa 939 waxy. It is quite probable that these differences are more apparent than real. If the differences in these biological values were real, then the biological value of the composite of corn germ (Experiment 13) should have been somewhat higher than 65 ± 1.3 . Differences in the rats used and their day-to-day condition, not always noted nor always shown by the probable errors of their biological values, frequently make differences of this magnitude nonsignificant. There were no significant differences between the average biological values of the corn germ proteins of the starchy and waxy inbreds in each line, namely, OS426 and I205.

Similarly, the data in Table II show that there were no significant differences between the average biological values of the corn endosperm proteins of OS426 and I205 whether one considers either the waxy or starchy varieties. These biological values, namely, 54, 59, 55, and

55, are so much alike that they might well have all been determined upon the same sample. This similarity in the nutritive value of these endosperm proteins serves to emphasize further the contention that the differences in the biological values obtained from the corn germ proteins of these inbred lines were more apparent than real.

TABLE II
UTILIZATION OF PROTEIN FROM CORN ENDOSPERM OF DIFFERENT GENETIC CONSTITUTION AS MEASURED BY MATURE RATS

Source of endosperm	Expt. no.	Rat nos.	Percentage protein ¹		Coeff. of true digestibility ³	Biol. value ²	Avg. wt. change of rats ²
			Endo-sperm	Ration			
			%	%	Avg.	Avg.	g.
OS426 waxy	17	57-68 ³	10.55	5.13	94 ± 1.3	54 ± 2.4	- 7.3
OS426 starchy	15	57-67	10.75	5.17	94 ± 1.4	59 ± 1.7	- 4.6
I205 waxy	18	69-79	11.34	5.02	91 ± 1.1	55 ± 1.3	-10.4
I205 starchy	19	69-79 ⁴	10.88	5.15	93 ± 1.1	55 ± 2.1	- 8.8
OS426 starchy ⁵	16	57-67	4.08	3.60	89 ± 1.2	70 ± 1.7	- 3.6
Corn gluten (Iowa 939 waxy)	6	23-33	42.22	5.23	95 ± 0.6	44 ± 1.9	- 9.2

¹ Protein = $N \times 6.25$.

² Computations based on a 7-day collection period following a conditioning interval of similar length. \pm = probable error.

³ Rats no. 59 and 60 became sick during Experiment 17. Rat no. 60 replaced by no. 68.

⁴ Rat no. 76 became sick. Discarded.

⁵ Ethanol extracted to remove most of zein.

The differences between the biological values of the samples of corn endosperm and the biological value of 44 ± 1.9 obtained for Iowa 939 waxy gluten (Experiment 6) are highly significant. The more starchy corn endosperm proteins constitute the original source of Iowa 939 waxy gluten utilized in Experiment 6. The endosperm sample contained much starch and it may be that corn gluten when encapsulated with its normal starch component has a somewhat higher biological value than when the starch is largely removed. Also, it is possible that in the removal of the starch from the gluten small amounts of more nutritionally complete protein are removed. The early work of Osborne and Mendel (6, 7) demonstrating the relative values of zein, corn gluten, and the corn glutelins would make such an explanation plausible. Finally, it is possible that in the process of separating the corn gluten from the starch, the gluten undergoes some degree of protein denaturation.

A sample of OS426 starchy endosperm which had been extracted with ethanol analyzed only 4.08% crude protein. Without further removal of carbohydrate it was impossible to introduce this ethanol-extracted endosperm into the basal ration in sufficient quantity to provide the rats with a diet containing 5% protein. Using the same basal ration as formerly (10) the test ration of ethanol-extracted

endosperm (Experiment 16) contained only 3.6% crude protein. This low percentage of protein may partly account for the rather high biological value of 70 established for this sample (Experiment 16). This value of 70, which is practically as high as the highest values obtained for corn germ reported in Table I, probably indicates that the residual proteins not extracted by the ethanol from the endosperm were of high quality. This may account in part for the high biological values obtained for these corn endosperm proteins, especially when compared to the biological value of the Iowa 939 waxy gluten used in Experiment 6. This result might have been predicted from the early work of Osborne and Mendel (6, 7) which demonstrated the superior value of the corn glutelins when compared to zein and corn gluten.

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COMPARISON OF CERTAIN CHEMICAL, PHYSICAL, AND BAKING PROPERTIES OF COMMERCIAL, BUHLER, AND HOBART-MILLED FLOURS¹

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ABSTRACT

Commercial, Buhler, and Hobart flours were extracted from subsamples of each of seven commercial lots of wheat, representing the classes soft red winter, hard red winter, and hard red spring. The three types of millings were compared with respect to flour yield, loaf volume potentialities, mixing and oxidation requirements, cookie spread factor and top grain, and other physical and chemical properties including granulation, baking absorption, gas production, and crude fat, ash, and protein contents. The Hobart grinder, in conjunction with the wheat roll electrodes of a Tag-Heppenstall moisture meter, can be used as a micro-mill for extracting flours having physical, chemical, and baking properties that are comparable to those for Buhler and commercial-milled flours.

One of the needs in the development of simple methods for evaluating the quality of small samples of wheat was fulfilled by the work of Finney and Yamazaki (5) who describe a simple and rapid micro-milling procedure utilizing a Hobart grinder in conjunction with the wheat roll electrodes of a Tag-Heppenstall moisture meter. Their paper describes the techniques used in obtaining flours having ash and protein contents comparable to those obtained by the usual experimental milling procedures. Questions pertaining to the normalcy of Hobart-milled flours with respect to certain physical, baking, and other chemical properties were reserved for a separate investigation that is reported at this time.

Materials and Methods

Commercial, Buhler-experimental, and Hobart-micro-experimental flours were extracted from subsamples of each of seven commercial lots of wheat representing soft red winter, hard red winter, and hard red spring classes. The following milling companies supplied commercially milled flour and subsamples of the grain from which extracted.

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³ Federal Soft Wheat Laboratory, Ohio Agricultural Experiment Station, Wooster, Ohio.

	Class of Wheat
Mennel Milling Co., Fostoria, Ohio	SRW
J. C. Lysle Milling Co., Leavenworth, Kansas	SRW
Midland Flour Milling Co., Kansas City, Mo.	HRW
Kansas Milling Co., Wichita, Kansas	HRW
Montana Flour Mills Co., Great Falls, Montana	HRW
Montana Flour Mills Co., Great Falls, Montana	HRS
Bay State Milling Co., Winona, Minnesota	HRS

All commercial-milled flours were straight grade, excepting the Bay State HRS which was a short patent. All Buhler and Hobart flours were straight grade.

The Buhler millings were made as described by McCluggage, Anderson, and Larmour (6). The procedures and techniques for Hobart-milling, bread and cookie baking, and the determination of absorption, dough mixing requirement, and bromate requirement are described by Finney *et al.* (5, 3, 7, 2, and 4). Gas production, crude fat, ash, and protein content were determined according to Cereal Laboratory Methods (1). Granulation was determined by sifting 50 g. of flour for one hour in a Ro-Tap shaker equipped with 115, 150, 200, and 270 mesh per inch Tyler screens. Carmichael cleaners were placed beneath each screen. Preliminary studies indicated the necessity, particularly on the commercial SRW flours, of sifting for one hour and of brushing off each screen every 15 minutes.

Results

Flour Yield. Flour yields for the commercial, Buhler, and Hobart milling procedures are given in Table I. The higher yields for the commercial flours compared to those extracted on the Buhler and Hobart mills is due, undoubtedly, to the long system and large quantity of material milled. It should be pointed out that the Hobart grinder is not regarded as a mill for characterizing wheats as to milling properties and potentialities, although the possibilities in this direction are good when in the hands of an experienced operator. Its chief value is for extracting representative flour samples from quantities of grain too small to mill satisfactorily in the Buhler or Allis experimental mills. Research and quality evaluation studies can be carried out on these flours using micro methods. Even for tests ordinarily requiring larger amounts of flour, limited but valuable information can be obtained.

Flour Ash. Flour ash contents for the commercial, Buhler, and Hobart-milled flours are shown in Fig. 1. The ash values for the Hobart flours closely parallel, but are consistently higher than, those for the Buhler flours. The obvious lack of correlation between the commercial flour ash contents and those for the Buhler and Hobart experimental flours is not regarded as being inconsistent, particularly

in view of the lack of comparability of the commercial millings. For example, the hard red spring flour from Bay State Milling Company was a short patent. This flour together with the Montana hard red

TABLE I
MILLING, CHEMICAL, AND PHYSICAL DATA FOR COMMERCIAL,
BUHLER, AND HOBART-MILLED FLOURS

Class and source	Coml.	Buhler	Hobart	Coml.	Buhler	Hobart
	FLOUR YIELD, %			FLOUR PROTEIN, %		
SRW —Mennel	69.4	62.0	63.6	7.9	8.0	8.1
SRW —Lysle	71.5	64.7	63.6	9.2	8.9	9.3
HRW—Montana	73.5	68.5	65.2	10.7	10.8	10.6
HRW—Midland	73.1	65.7	63.4	11.2	11.0	11.2
HRW—Kansas	73.5	66.0	63.9	11.0	10.9	11.3
HRS —Montana	72.2	64.2	63.5	13.1	13.6	14.4
HRS —Bay State	58.0 ¹	63.3	64.7	12.1 ¹	12.1	13.2
	CRUDE FAT, %			ABSORPTION (BREAD BAKING) %		
SRW —Mennel	1.1	0.9	1.2	51.0	50.5	50.5
SRW —Lysle	1.0	0.9	1.2	58.5	56.0	58.0
HRW—Montana	1.1	1.0	1.2	60.0	58.5	58.5
HRW—Midland	1.3	1.0	1.2	65.0	63.5	64.0
HRW—Kansas	1.1	0.9	1.0	67.5	64.5	65.0
HRS —Montana	1.2	1.0	1.5	63.5	63.0	64.0
HRS —Bay State	1.1 ¹	1.0	1.4	64.5 ¹	61.0	62.0
	MIXING REQUIREMENT, MIN.			BROMATE REQUIREMENT, MG.		
SRW —Mennel	2 $\frac{5}{8}$	2 $\frac{5}{8}$	2 $\frac{5}{8}$	2	3	2
SRW —Lysle	3	2 $\frac{7}{8}$	3	2	2	3
HRW—Montana	1 $\frac{3}{4}$	1 $\frac{3}{4}$	1 $\frac{5}{8}$	6	6	6
HRW—Midland	2 $\frac{7}{8}$	3	3 $\frac{1}{4}$	3	3	3
HRW—Kansas	3	2 $\frac{7}{8}$	2 $\frac{7}{8}$	4	4	4
HRS —Montana	3 $\frac{3}{4}$	3 $\frac{1}{2}$	3 $\frac{1}{4}$	2	2	2
HRS —Bay State	3 $\frac{1}{2}$ ¹	3	3	2 ¹	2	2

¹ Short patent—all others straight grade.

spring and the two soft red winter flours were lower in flour yield to varying degrees than the three hard red winter commercial-milled flours, thereby accounting for lower ash values.

Flour Protein and Crude Fat. Values for these tests are given in Table I. Crude fat indicates the relative amounts of wheat germ extracted by the three milling procedures. Both protein and crude

fat contents for the commercial, Buhler, and Hobart millings are in good agreement for the soft and hard red winter classes. For the hard red spring wheat flours extracted on the Hobart grinder, however, protein contents averaged about 1% higher and crude fat values about 0.4% higher than the corresponding values for commercial and Buhler millings. The extreme hardness of the two hard red spring wheats probably accounts for the relatively high protein and crude fat contents of the two corresponding Hobart-milled flours. These probably could be reduced by making minor changes in the milling procedure.

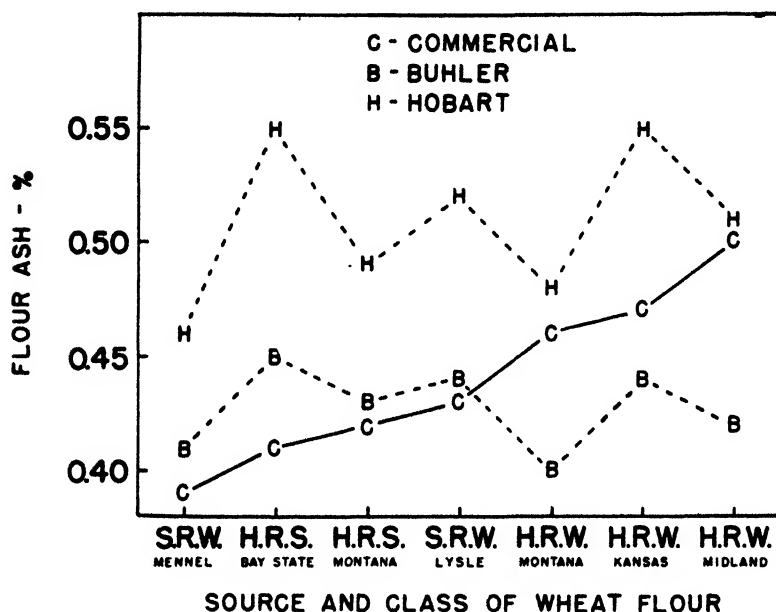


Fig. 1. Flour ash for commercial, Buhler, and Hobart-milled flours.

Granulation. Comparative granulation data obtained on the three millings representing each of the seven commercial wheats are given in Table II. The results are quite consistent in that a nearly negligible quantity of flour remains over the 115-mesh sieve for the commercial and Buhler flours in contrast to an average of 4.1% for the Hobart millings. Similarly, the overs of the 150-mesh sieve for the Hobart millings are considerably greater than for the two other millings. The greater amounts of overs of the 115- and 150-mesh sieves for the Hobart millings account, to a large extent, for the appreciably smaller amounts of material over the 200- and 270-mesh sieves compared to the commercial and Buhler millings. These granulation differences undoubtedly are accounted for largely by the differences between the extrac-

tion systems and flour sieves used for the three mills. In contrast to the long commercial system, the Buhler millings were made with three breaks and three reductions, and those on the Hobart with only one break and two reductions. Where the equivalent of a 9XX flour cloth was used in the Hobart procedure, 10XX flour cloths were used on the Buhler, and 10XX to 11XX cloths were used for the commercial

TABLE II
GRANULATION OF COMMERCIAL, BUHLER, AND HOBART-MILLED FLOURS

Sample	Overs 115	Overs 150	Overs 200	Overs 270	Throughs 270
	%	%	%	%	%
<i>Mennel—S.R.W.</i>					
Coml.	0.0	0.2	10.8	19.4	62.8
Hobart	1.0	10.0	15.2	8.4	60.0
Buhler	0.2	2.0	27.0	14.8	52.4
<i>Lysle—S.R.W.</i>					
Coml.	0.2	2.0	23.2	18.6	51.0
Hobart	2.8	15.6	19.4	11.0	45.4
Buhler	0.0	2.8	32.6	16.6	45.0
<i>Bay State—H.R.S.</i>					
Coml.	1.4	11.6	29.4	19.4	34.6
Hobart	4.4	20.0	24.6	14.0	33.0
Buhler	0.0	6.2	41.6	17.2	31.4
<i>Montana—H.R.S.</i>					
Coml.	0.2	4.2	27.4	22.2	43.0
Hobart	4.4	19.2	23.6	12.2	36.2
Buhler	0.0	5.4	40.8	18.4	31.8
<i>Montana—H.R.W.</i>					
Coml.	0.2	5.8	32.8	19.8	39.6
Hobart	4.8	19.6	22.6	11.8	36.6
Buhler	0.0	3.8	39.0	19.8	33.0
<i>Midland—H.R.W.</i>					
Coml.	0.2	1.6	22.2	23.2	49.6
Hobart	5.6	20.6	23.0	9.8	38.0
Buhler	0.0	3.2	35.8	20.2	37.0
<i>Kansas—H.R.W.</i>					
Coml.	0.2	3.8	26.0	20.0	47.6
Hobart	5.8	20.4	23.0	13.2	36.2
Buhler	0.0	4.4	38.2	19.0	35.4

millings. It is pertinent to note, however, that the throughs of the 270 for the Hobart flours are consistently greater than those for the Buhler millings by an average of 2.8%. Considering the differences in the three mills, the average granulations for the three types of millings are surprisingly similar.

4th Hour Gas Production. Gassing data for the seven soft and hard wheat flours extracted by each of the three milling procedures are

shown graphically in Fig. 2. The results for the Buhler and Hobart millings are nearly identical and obviously are highly correlated with those for the commercial flours. These data are in accord with the granulation data, since they indicate that the Buhler and Hobart millings have about the same average flour particle size, and are appreciably coarser than the commercial flours.

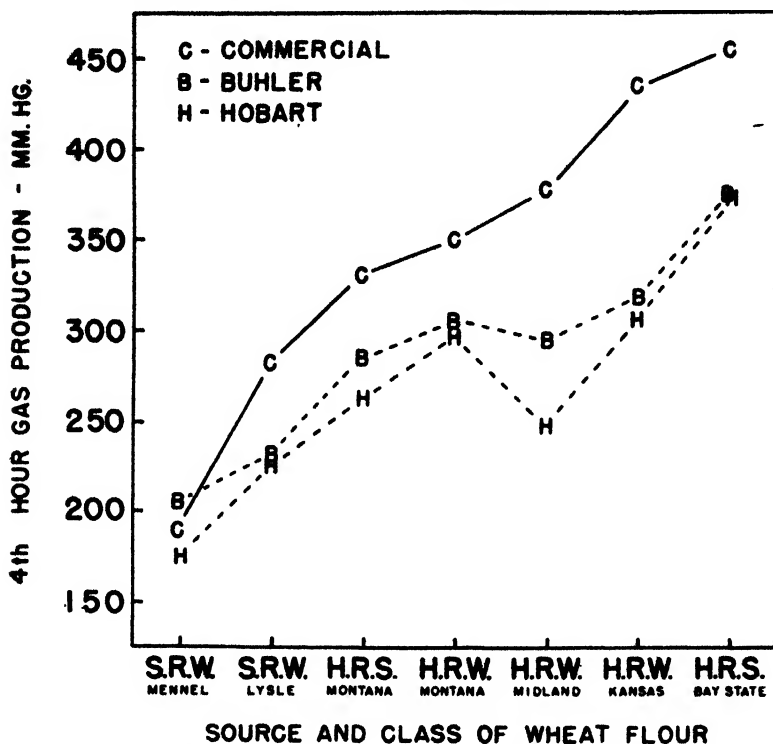


Fig. 2. Fourth hour gas production for commercial, Buhler, and Hobart-milled flours.

Absorption, Mixing, and Bromate Requirements. A study of the relation of milling procedure or technique to absorption, mixing, and bromate requirements is warranted because of their importance in baking technology and the production of good bread. The data for baking absorption, dough mixing time, and potassium bromate requirement are given in Table I. It is apparent from an examination of these data that, for a given test, a very high correlation exists between the results for any two milling procedures. As a matter of fact, the variation due to milling method is practically negligible for mixing and bromate requirements. The somewhat higher absorption, in general, for the commercially extracted flours is probably due to their finer particle size.

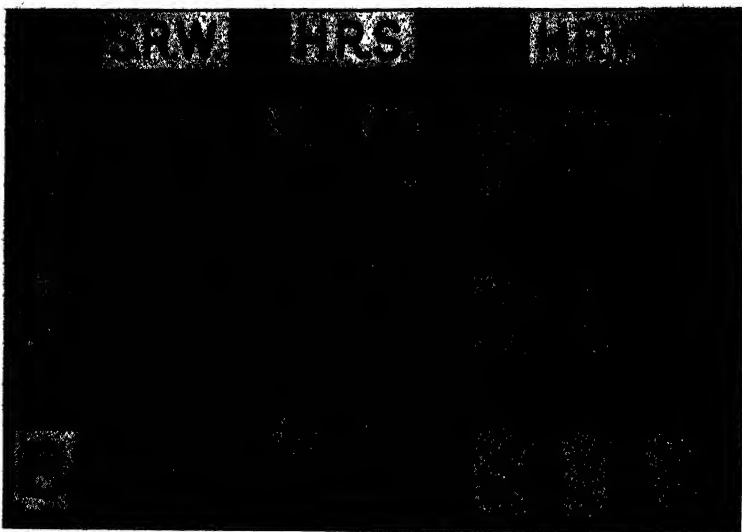


Fig. 3. Loaves of bread baked from commercial, Buhler, and Hobart-milled flours. Source of flour (left to right): Mennel, Lysle, Bay State, Montana, Montana, Midland, Kansas.

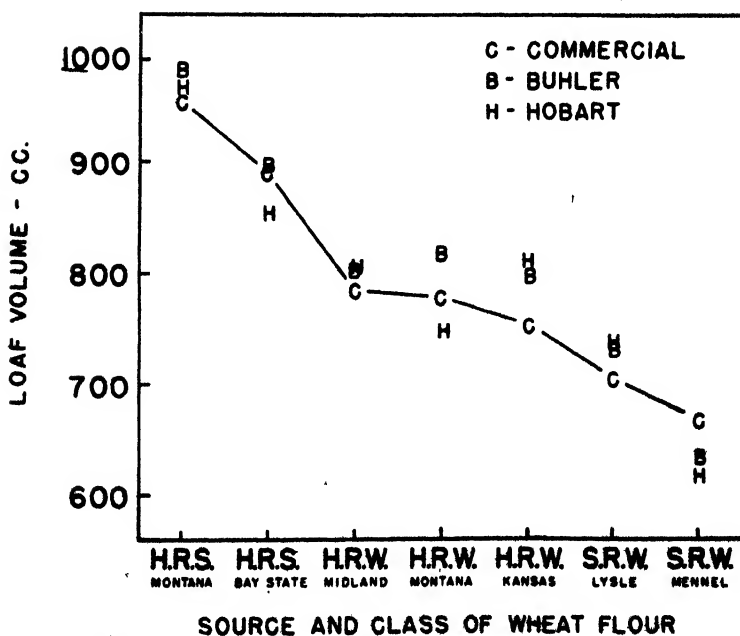


Fig. 4. Loaf volume for commercial, Buhler, and Hobart-milled flours.

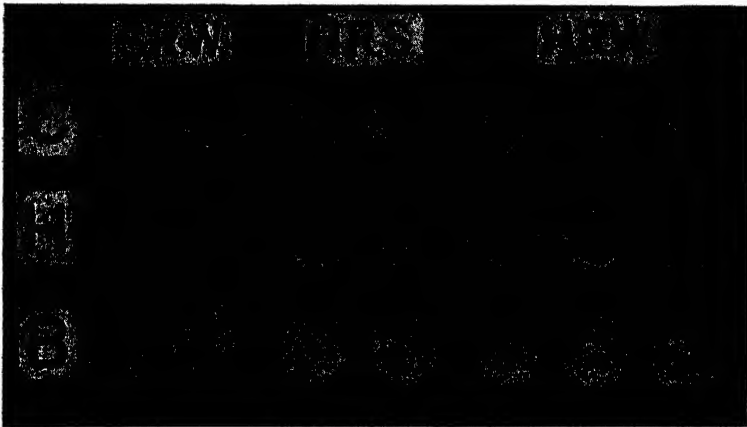


Fig. 5. Sugar snap cookies baked from commercial, Buhler, and Hobart-milled flours. Source of flour (left to right): Mennel, Lysle, Bay State, Montana, Montana, Midland, Kansas.

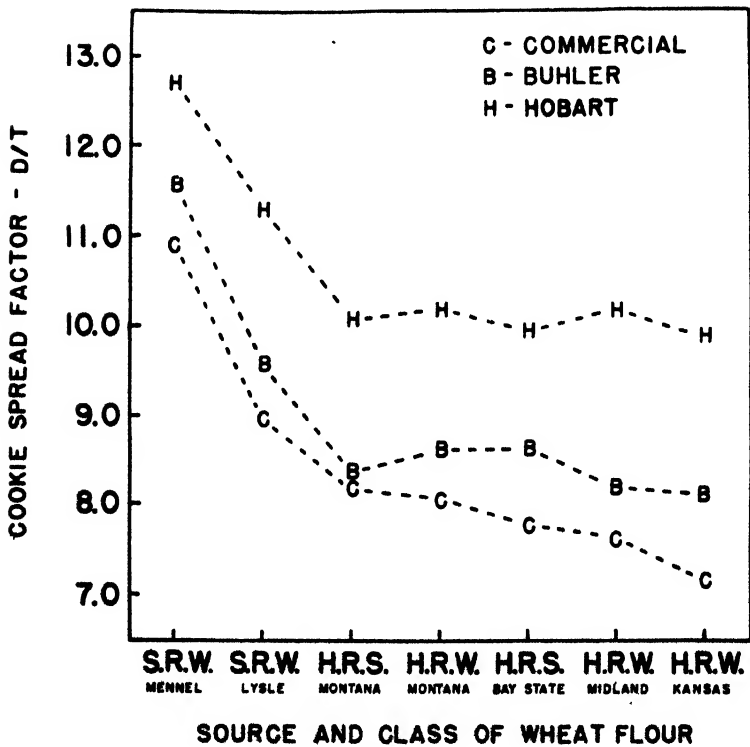


Fig. 6. Cookie spread factor (diameter/thickness) for commercial, Buhler, and Hobart-milled flours.

Loaf Volume of Bread. Internal characteristics and relative size of loaves of bread representing the seven different wheats and three mills are shown in Fig. 3. Loaf volumes are shown graphically in Fig. 4. These figures show that flours extracted on the Hobart grinder will yield bread having crumb grains and loaf volumes equal or very similar to those for flours extracted on the Buhler and commercial mills.

Cookie Spread Factor (D/T). Top grains and relative diameters for cookies baked from the 21 flours used throughout these studies are shown in Fig. 5. Cookie spread factors (diameter/thickness) are presented graphically in Fig. 6.

The well-broken top grain and greater cookie spread factor produced from the Hobart flours indicate superior cookie baking quality. While this superiority of the Hobart flour cookies may be partially attributed to differences in particle size distribution, nevertheless the spread factors (Fig. 6) for the Hobart-milled flours are highly correlated with those for the commercial and Buhler-milled flours. This is evident since the two soft winter flours are ranked in the same order and above the hard winter and spring flours for all three milling procedures.

Acknowledgment

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EFFECTS OF MIXING, SALT, AND CONSISTENCY ON EXTENSOGRAMS¹

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ABSTRACT

Studies of the effects of initial mixing procedures on extensograms were made with the Hobart, Brabender Farinograph, and Swanson mixers. With the mild mixing action of the Hobart, little change in extensibility (length) or resistance to extension (height) occurs as mixing time is increased, and there is little difference between 45-minute and 135-minute extensograms. With the severe mixing of the Swanson, extensibility decreases and resistance increases with increasing mixing time, and these changes are greater in 135- than in 45-minute extensograms. The farinograph mixer gives intermediate results. Mild mixing is considered to yield extensograms representing inherent properties of the flour and to provide a control procedure for studies of changes in formula or manipulations. Severe mixing yields extensograms from which the response of the flour to mixing can be measured by the differences between 45- and 135-minute curves. Flours from a wide range of wheats differ greatly in both basic properties and in response to mixing.

Increasing the consistency of the dough decreases extensibility and increases resistance. By contrast, increasing the salt concentration increases both extensibility and resistance. Salt also changes the shape of the curve by moving the highest point towards the right. Weak and strong flours respond differentially to added salt, so that comparisons of extensibility depend upon whether this is measured by the total length of the extensogram or by the length to the highest point (greatest resistance). These two measurements are discussed in terms of their probable relation to elastic and viscous deformation during stretching.

As a basic procedure for extensograph tests, the authors have adopted the salt concentration, consistency, and mixing procedure used in their test baking.

Tests made with the Brabender Extensograph can be varied in technique as widely as the baking test. Absorption, formula, type of mixer, and time of mixing can be readily changed. The schedule of dough manipulations, which corresponds to punching in the baking test, can be varied for number, timing, and kind of treatment.

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Changes can be made in the conditions under which the doughs are rested. The extensograph mechanism can be adjusted to give extensograms of different heights, and it would not be difficult to change the method of stretching in other ways. Various curve dimensions can be measured, and these can be reported in different units. Great scope clearly exists for varying the technique, and it is difficult to select the best procedure for given purposes.

The technique recommended by the Brabender Corporation in the Manual of Instructions appears to represent a relatively arbitrary selection. Munz and Brabender (5, 6, 7) did not use the recommended procedure in any of their comprehensive studies. They employed a wide range of techniques, usually tested several replicate doughs treated in different ways, and generally condensed their data by such methods as plotting the F/E ratio (height/length) against the logarithm of rest period. These investigations have little bearing on the development of a simple and rapid extensograph test. Indeed, the inescapable conclusion appears to be that a large number of extensograph tests are required to obtain useful information about a flour, and that more comprehensive and perspicuous data may well be provided by devoting an equal effort to traditional baking studies.

Other investigators (1, 3, 4) have also deviated from the manufacturer's instructions, and private communications and observations made during visits to various laboratories, on this continent and in the United Kingdom, suggest that a wide range of techniques is now in use. If all the information about varying the technique were published and could be collated, substantial progress in defining the best methods for using the extensograph might well result. This paper is published with that goal in mind.

In this laboratory, the purpose has been to devise the simplest extensograph test or tests that will yield interpretable data on the innate properties of flour. Provided that tests can be made rapidly, the extensograph may well have widespread uses for control in mill and bake-shop, and possibly as a tool for research. Development along either line is inhibited by inadequate information about rudimentary technique. Without this knowledge, it is difficult to find a logical point of departure for more advanced studies or adequate knowledge for interpretation of data that such studies may yield.

This paper presents results of studies of mixing, salt concentration, and consistency. These factors were investigated with flours made from a series of wheats representing a wide range of types so that differential responses of type to change in technique might be demonstrated. Although large numbers of data were accumulated, only sufficient need be presented to illustrate the points discussed. A brief des-

cription of the materials is given in the following section. Separate sections are then devoted to a study of mixing and a study of the effects of varying salt concentration and consistency. As a result of these investigations, certain basic techniques and concepts have been adopted in this laboratory and are discussed in the final section.

Materials

Descriptions of the materials used in these studies are of little importance since any other series of wheats exhibiting a similar range in quality would have been equally suitable. Sixteen wheats of different classes were obtained from various countries, but it is not claimed that all samples represent the average of their class. The wheats were cleaned, and were then milled in an Allis-Chalmers laboratory mill to yield long patent flours of about 71% extraction. The wheats represented and the protein contents of the flours are listed below:

<i>Ref. letter</i>	<i>Wheat</i>	<i>Flour protein, %</i>
A	High protein Canadian (4 Northern)	16.1
B	Cadet variety (grown in Canada)	14.1
C	Rescue variety (grown in Canada)	13.6
D	Marquis variety (grown in Canada)	13.5
E	No. 1 Northern (average sample)	13.0
F	Amber durum	12.1
G	Garnet variety (grown in Canada)	10.9
H	Plate (Argentine semihard winter)	10.7
I	Kansas hard winter	10.5
J	Alberta red winter	10.3
K	Kansas soft winter	10.1
L	Pacific (U. S.) soft winter	9.7
M	Australian	9.3
N	English	9.2
O	Ontario white winter	9.0
P	Low protein Canadian (3 Northern)	8.6

Effects of Mixing

The manufacturers specify that doughs for extensograph tests be mixed in the farinograph mixer. In a preliminary trial, the amount of water added to a flour containing 2% salt is adjusted so that the center of the farinogram reaches the 500-unit line when the curve attains its maximum height. After adding the required amount of water, the dough for the extensograph test is mixed for 1 minute, rested for 5 minutes, and then mixed until the center of the curve just reaches the 500-unit line. This is variously referred to, by different authors, as mixing to minimum mobility, mixing to maximum dough development, or mixing to dough development time.

Johnson, Shellenberger, and Swanson (3) followed these instructions. Merritt and Bailey (4) varied them by omitting the 5-minute rest period. But Munz and Brabender himself (5) apparently mixed

all flours for 5 minutes, except in their study of extended mixing. Aitken, Fisher, and Anderson (1) also mixed for 5 minutes, but worked at a dough consistency represented by the 600- rather than the 500-unit line. The selection of the farinograph for mixing doughs for extensograph tests appears to be an arbitrary one for which the literature contains no support.

Experimental. Studies were made by mixing doughs for 1, 2, 3, and 4 minutes in the farinograph, the Hobart (medium speed), and the Swanson mixers, using a salt concentration of 2% and a dough consistency of 500 units. After mixing, two 100-g. doughs were scaled, rounded and rolled in the extensograph, rested for 45 minutes at 30°C. and 85% relative humidity, and subsequently stretched. Doughs were

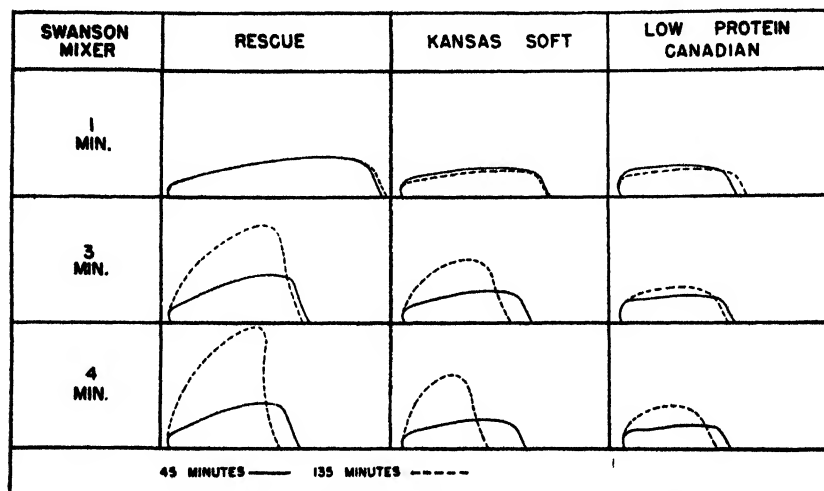


Fig. 1. Extensograms (45 min. solid, 135 min. dotted) showing effects of mixing doughs in the Swanson mixer for different times.

rounded and rolled a second time after 45 minutes but were not stretched. After a further 45 minutes, the doughs were stretched a second time. Extensograms were thus made 45 and 135 minutes after mixing; but the second curve, like the first, was also made 45 minutes after the last rounding and rolling. Six flours were studied, but a selection of the results is adequate to illustrate the principles established.

Because the Swanson mixer has the most severe action on the dough, results obtained with it are exaggerated the most. Reproduction of the extensograms for three of the samples (Rescue, Kansas soft winter, and low-protein Canadian) are shown in Fig. 1. With the 45-minute curve (solid) increasing the mixing time decreases length

and increases height, and this effect is greater for Rescue than for the other two flours. Similar but still greater effects are shown by the 135-minute curves (dotted). Thus, the difference between 45- and 135-minute curves increases with increasing initial mixing, and this response is greatest for the first flour, intermediate for the second, and least for the third.

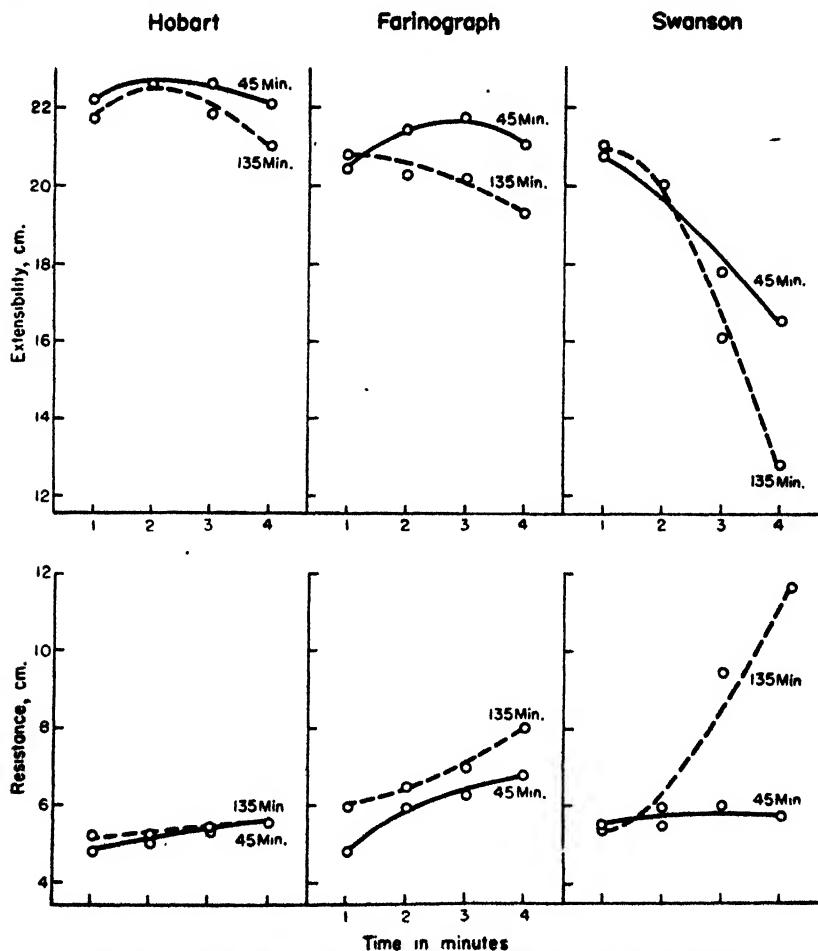


Fig. 2. Graphs showing effects on extensogram measurements of mixing doughs in different mixers. The data are mean values for flours C, E, G, K, L, and P.

These results cannot readily be compared with those of Munz and Brabender (5) because they reported resistance and extensibility as a ratio plotted against the logarithm of rest period. However, their study of extended mixing certainly demonstrated a differential response of flours to mixing time.

The differential effects of the three mixers are illustrated by the graphs in Fig. 2. Extensibility and resistance at 45 minutes (solid lines) and at 135 minutes (dotted lines) are plotted against mixing time, for the mean data obtained with the six flours, C, E, G, K, L, and P. For the Hobart mixer, which has the mildest action, extensogram dimensions remain closely similar for both rest periods as mixing time increases. The farinograph mixer, which has more severe action than the Hobart mixer, shows an increased spread between extensogram dimensions with increased mixing time, and this is exaggerated by the much greater severity of the Swanson mixer. Optima in the upper 45-minute curves for the Hobart and farinograph mixers are interesting; apparently the dough is most extensible when mixed in either mixer for 3 minutes. With the Swanson mixer, maximum extensibility was obtained with minimum mixing time.

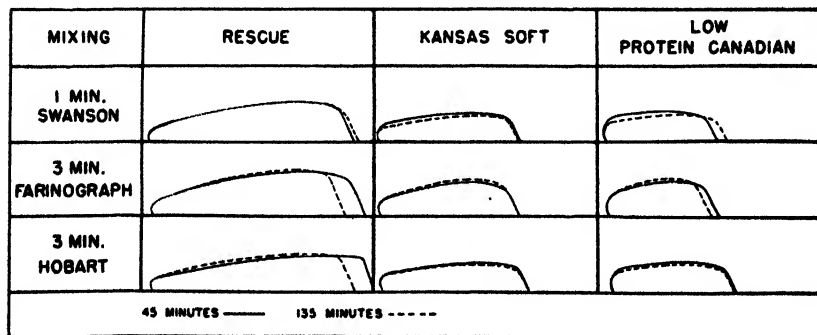


Fig. 3. Extensograms (45 min. solid, 135 min. dotted) showing effects of mixing doughs in different mixers when about the same amount of mixing is done on the dough.

Provided that about the same amount of mixing is done on the dough, the type of mixer is probably unimportant. Fig. 3 shows that 45-minute extensograms afford essentially the same comparisons between the same three flours represented in Fig. 1, irrespective of whether the dough is mixed for 1 minute in the Swanson mixer or for 3 minutes in either the farinograph or the Hobart mixer. Moreover, with these amounts of mixing, the 135-minute extensograms are not widely different from the 45-minute extensograms, though there are some differences which cannot be accounted for merely by experimental error and must therefore be attributed to the mixing.

Mixing to dough development time in the farinograph mixer is compared in Fig. 4 with mixing for 3 minutes in the Hobart mixer using flours A, E, H, P, I, and G. That the three flours in the upper half of the figure exhibit considerable response to mixing is shown by the differences between the 45-minute and the 135-minute extenso-

grams in the top row; farinograph mixing times for these three samples, from left to right, were 6, 6, and $2\frac{1}{4}$ minutes. When the same samples were mixed for 3 minutes in the Hobart mixer, the first two show their response to mixing by a shorter extensogram at 135 minutes, whereas the extensogram for the Plate flour (H) at 135 minutes shows a decrease in height. The lower half of the figure shows extensograms for three flours that have very little response to mixing. Farinograph mixing times for these flours, from left to right, were $1\frac{3}{4}$, 3, and 2 minutes. Regardless of whether these three doughs were

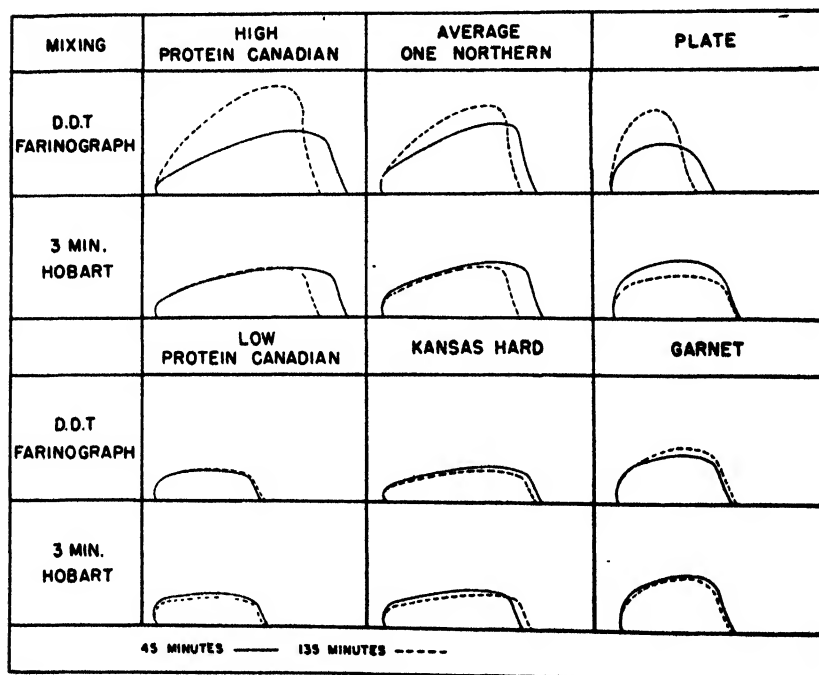


Fig. 4. Extensograms (45 min. solid, 135 min. dotted) showing effects of mixing to development time in the farinograph and for 3 min. in the Hobart mixer.

mixed for a variable time in the farinograph mixer or for 3 minutes in the Hobart mixer, extensograms for 45 minutes and 135 minutes are essentially the same.

Discussion. Whether it is best to vary mixing to meet the requirements of individual flours or to mix all flours equally is debatable. If variable mixing is adopted in accordance with the manual, the treatment should presumably be optimum for each flour, and this supposes that an estimation of optimum treatment, preferably an objective one, is available. Mixing to dough development time in the farinograph

mixer does provide an objective test; but it is questionable whether this procedure assesses optimum mixing treatment for each flour. Observation suggests that some doughs are overmixed. If there is no assurance that each dough can be mixed to given optimum mixing, then all doughs may well be given equal mixing treatment. Otherwise, there is no way of knowing whether results wholly represent intrinsic differences between flours, or are biased by differences in the accuracy with which optimum mixing conditions have been selected.

When all flours are mixed in the same way, the interpretation of the resulting extensograms depends on the severity of the selected mixing method. A distinction may usefully be made between the basic properties exhibited by the dough after mild mixing and the properties the dough may develop through its response to more severe mixing. Mild mixing may be defined as an amount that causes minimum changes in dimensions between 45- and 135-minute extensograms. Results for a wide range of flours suggest that this criterion is met by mixing for 3 minutes in the Hobart mixer. By adjusting the mixing time, other mixers with relatively mild actions will doubtless give similar results. Since 45- and 135-minute curves are essentially the same, only the first need be prepared when this technique is used. Data on extensibility and resistance thus obtained may be considered to represent the basic properties of the flour. The technique thus provides a control procedure with which the effects of modifying manipulative procedure or formula may be compared.

By increasing the time or severity of the initial mixing, the extensograph can be used to measure response to mixing. This response is more pronounced in the 135-minute extensograms. A strict comparison of mixing responses might thus involve comparison of 135-minute extensograms made with the basic procedure and with severe mixing. For many purposes, however, the difference between 45- and 135-minute extensograms made on one dough that has been severely mixed provides an adequate comparative measurement of mixing response.

When the extensograph is used to study the effects on dough properties of some added ingredient, mild mixing may well prove best. For instance, the bromate response, which takes some time to develop, can be measured with 135-minute extensograms. If the mixing is mild, the effect of bromate is negligible after 45 minutes, and the curve thus drawn represents the basic dough properties. Accordingly, the difference between the 45- and 135-minute curves represents the effect of bromate. But if the mixing is severe, the difference between the curves represents a combination of mixing response and bromate response.

As extensograms are normally made at a standard dough consistency, some means of determining the absorption that gives this consistency is required; and the farinograph is extremely convenient for this purpose. But there appears to be no other reason why the farinograph should be used to mix doughs for extensograph tests. For many purposes, the mixer normally used for test baking, or a mixer that closely simulates commercial operations, will represent a more logical choice.

Effects of Varying Salt Concentration and Consistency

The prescribed technique for extensograph tests recommends 2% of salt and a consistency of 500 farinograph units. Except for the use of 600 units for consistency in one investigation (1), this procedure seems to have been generally adopted. Since the salt concentration is higher and the consistency lower than levels generally used in test

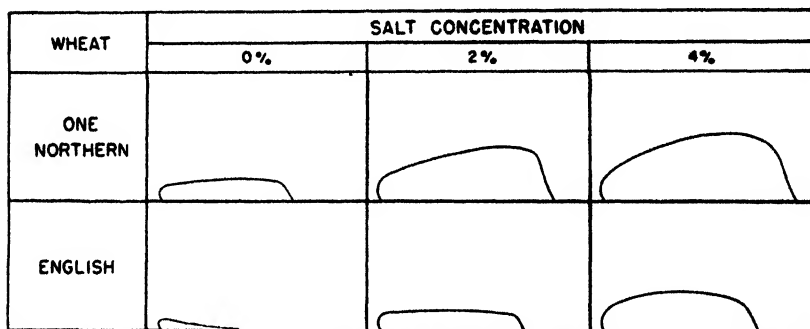


Fig. 5. Extensograms (45 min.) showing effects of varying the salt concentration in doughs.

baking, the selection seems to be arbitrary. No data on the effects of varying either factor have come to the authors' attention, and a study of the matter was therefore undertaken.

Experimental. Preliminary information on salt concentration was obtained from extensograms made with a strong Canadian flour (E) and a weak English flour (N). By the baker's tactile judgment, these two flours exhibited extremes in dough-handling properties. Consistency was adjusted to 540 units, a level which corresponds to that used in this laboratory for baking tests. Salt concentrations were 0, 2, and 4%, and doughs were mixed for 3 minutes in the Hobart mixer. Fig. 5 shows the extensograms made 45 minutes after mixing.

With both flours, increasing the salt concentration increases not only the resistance but also the extensibility. In the extensograms for the English flour the high point of the curve is near the beginning for no salt and moves towards the center as salt concentration is in-

creased. The shape of the extensogram for Canadian flour also changes with salt concentration by becoming progressively steeper. If extensibility is judged by the total length of the curve (E), the Canadian dough is nearly twice as extensible as the English with no salt; but with 2 and 4% of salt, differences in extensibility are considerably reduced. If extensibility is judged by the length of the curve to its highest point (E_1), the Canadian dough is more than 10 times as extensible as the English dough with 0 and 2% salt, and nearly three times as extensible with 4% salt. (Fig. 9 illustrates the dimensions E and E_1 .)

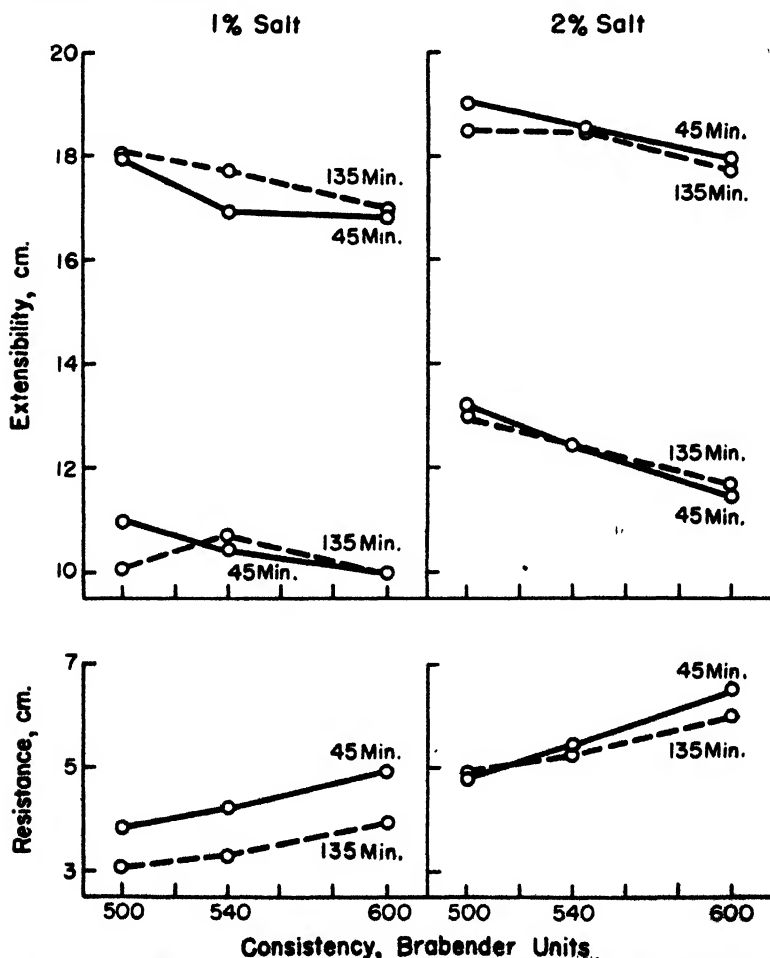


Fig. 6. Graphs showing effects on extensogram measurements of varying dough consistency and salt concentration. The data are mean values for flours E, F, H, I, O, and P. In top half of figure, upper lines represent total curve length (E) and lower lines represent curve length to maximum height (E_1).

In a larger study involving both salt concentration and consistency, extensograms were made from flours E, F, H, I, O, and P. Salt concentrations were 1% and 2%, and consistencies were 500, 540, and 600 units. The doughs were mixed for 3 minutes in a Hobart mixer, and extensograms were made 45 minutes and 135 minutes after mixing. The mean absorptions corresponding to these consistencies were 57.8, 56.8, and 55.1% with 1% of salt, and 58.5, 57.5, and 55.3% with 2% of salt.

Mean results are summarized for all flours in Fig. 6. Comparison of the data for the 45-minute extensograms (solid lines) with those for the 135-minute extensograms shows little difference between them. Since the mixing was mild, this was to be expected. Comparison of the curves on the left with those on the right shows that increasing the salt concentration increases extensibility and resistance. But the slopes of the curves show (with one exception) that increasing consistency decreases extensibility though it also increases resistance.

In the upper graphs, curves are given for both E (total curve length) and E_1 (length to greatest height). Both measurements react similarly to changes in salt concentration and consistency.

Data for 135-minute extensograms for four of the flours (E, P, F, and O) are plotted in Fig. 7. These curves are shown primarily to emphasize the differences between total extensibility (E) and extensibility to maximum height (E_1). Both with 1% of salt (top graphs) and with 2% of salt (bottom graphs) the spreads between curves for E and E_1 increase from flour E through flours P and F to flour O. Moreover, this generalization holds at all three levels of consistency. Accordingly, judgment of the comparative extensibilities of the flours will depend upon which measurement is made. For instance, the first and last flours are of about the same extensibility as judged by total extensogram length (E), but differ widely if judged by length to greatest height (E_1). Again, by the first measurement, flour O is more extensible than flour F, whereas O is less extensible if judged by the second measurement.

The 45-minute extensograms for these four flours are shown in Fig. 8 for consistencies of 500 and 600 units. Differences in the shapes of the extensograms are considerable. Average 1 Northern (E) yields curves with the highest point well towards the right, and the difference between the two extensibility measurements, E and E_1 , is thus comparatively small. For the low protein 3 Northern (P), the point of maximum height varies in position with the amount of salt; with 1% salt it is towards the left, and with 2% it is towards the right. With both Amber Durum (F) and Ontario white winter (O) the point of maximum height is invariably towards the left, and with these flours

E and E_1 differ widely. On the basis of total extensibility (E), little difference exists between the top and bottom flours in Fig. 6; but on the basis of extensibility to maximum height (E_1), the top flour would be judged to be far superior. Accordingly, the significance of an extensogram cannot be defined merely in terms of length, height, and area; attention must also be paid to its shape, although this can probably

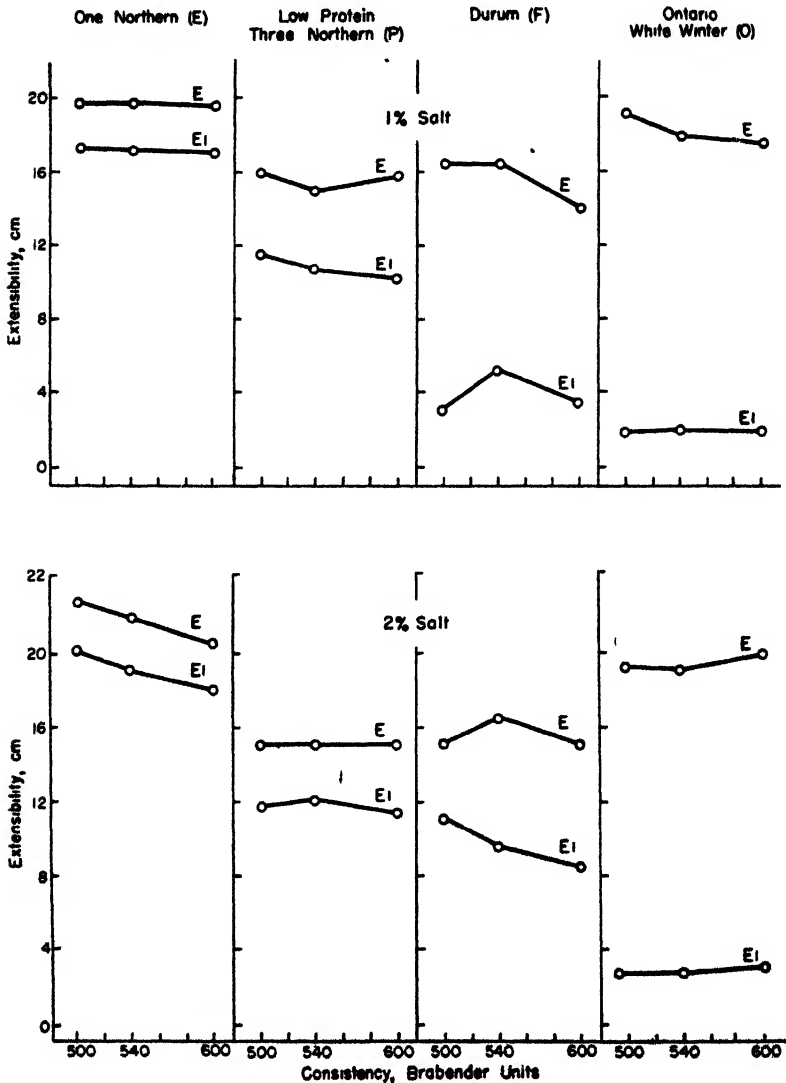


Fig. 7. Graphs for four flours showing effects on extensogram (135 min.) measurements of varying dough consistency and salt concentration.

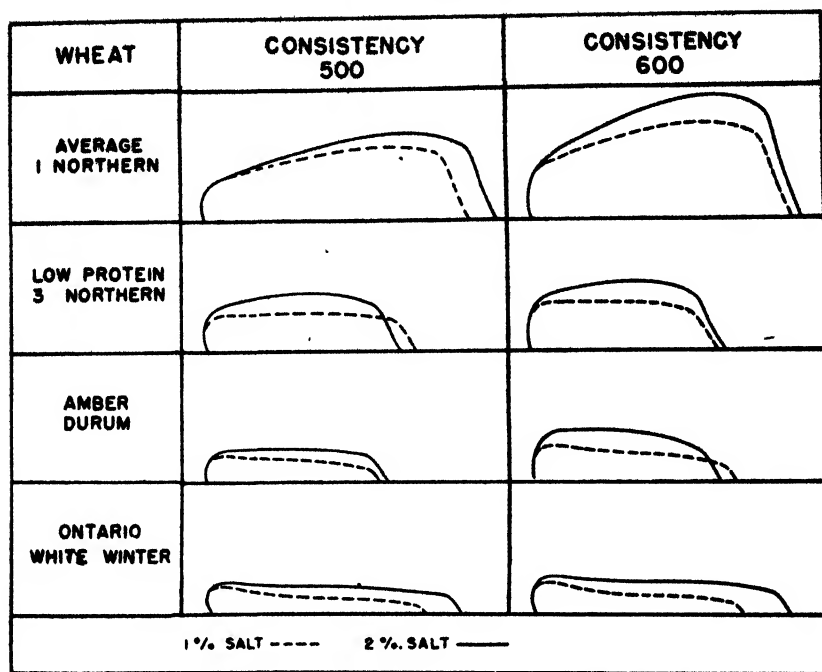


Fig. 8. Extensograms (45 min.) for four flours showing effects of different dough consistencies and salt concentrations.

be recorded for most purposes in terms of the measurements of E and E_1 .

Discussion. The main question arising from these studies of salt concentration and consistency relates to the measurement of extensibility. Merritt and Bailey (4) have advocated that E_1 rather than E should be measured because the former is more reproducible. But it also appears that these two measurements represent different properties or combinations of properties of the flours. If this is true, a technique which magnifies differences between the two measurements may well prove useful.

The fundamental properties responsible for the behavior of a dough in the extensograph are elasticity and viscosity. Since both properties are involved throughout most of the stretching, the extensograph cannot be expected to provide independent measurements of each. Nevertheless, the shape of the extensogram does appear to indicate the relative importance of these two properties at various stages of stretching.

The mechanism of stretching must be somewhat as follows. Cohesive forces within the resting dough require that a certain stress be imposed before appreciable stretching can begin, and there is thus a

sharp immediate rise in the curve. An extension of the three-dimensional network of the fibrillar molecules of the gluten complex must then occur. As stretching is continued, the network of molecules within the gluten strands, and the strands themselves, are extended. The elastic limit is probably exceeded at various points distributed at random throughout the dough, and the cohesive forces between certain molecules are broken. This period appears to be represented in the extensogram by a continued rise in the curve. Throughout this process of elastic deformation, there must also be viscous deformation

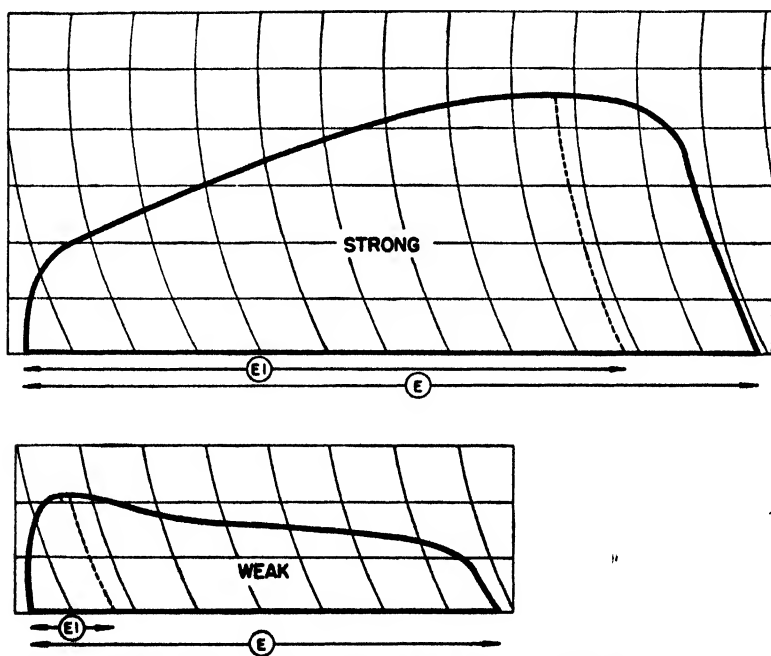


Fig. 9. Extensograms for strong and weak flours illustrating the measurement of E and E_1 .

of the dough, which also affects the shape of the curve. When all elasticity has been overcome, plastic flow appears to continue and the curve tends to fall. Finally, the dough strand starts to tear as its tensile strength is exceeded and the curve begins to drop sharply. If strands on both sides of the hook tear at the same time, the drop in the curve is abrupt; but if one strand tears before the other, a more gradual drop occurs.

If dough had no elastic properties, the initial rise would be followed by a gradual decline in the curve representing the decreased resistance to viscous deformation as the strand became thinner with elongation. If the dough were entirely elastic, like a spring, a straight line, with

slope proportional to the stiffness of the spring, would be drawn. The extensogram represents a compromise between these two extremes.

This hypothetical account of the possible mechanisms of stretching leads to a speculative interpretation of the shape of extensograms. Representative curves for a strong and a weak flour are shown in Fig. 9. In both curves, the elastic properties are considered to offer resistance to stretching until the curve starts to drop, i.e., until the highest point is reached. The remaining part of the curve is considered to represent continued viscous deformation. Accordingly, the measurement E_1 probably reflects the elastic properties of the dough much more than the measurement E . The curve for the strong flour indicates a "bucky" dough, since some elasticity is retained throughout the stretching almost to the point at which breaking of the strands occurs. The curve for the weak flour indicates a dough that has little elasticity (E_1 is short) but is soft and reasonably extensible.

Results reported earlier in this section indicate that increasing the consistency of the dough makes the extensogram higher and shorter but has comparatively little effect on the relations between E and E_1 . But increasing the salt concentration does appear to increase E_1 more than E and thus tends to mask differences in the elasticity of the flours. Increasing consistency, by reducing water content, probably affects the curve largely by increasing resistance to viscous deformation. But the salt appears to affect elasticity, presumably through action on the protein involving the Donnan effect.

In laboratories restricting studies to one type of flour, the levels of salt and consistencies selected for standard tests are probably of little consequence. All selections will place the flours in essentially the same rank order with respect to either measurement of extensibility. Indeed both measurements, E and E_1 , will prove to be highly correlated. But, in laboratories dealing with a wide range of flour types, some advantage may be gained from selecting a technique that emphasizes the differences between E and E_1 . For among different classes of flour, ranging from strong to weak, these two measurements are not correlated.

General Discussion

As a result of these studies, a basic technique has been adopted for extensograph tests made in this laboratory. It corresponds in usefulness to the basic baking formula. Thus it provides some information about the comparative qualities of different flours, but also serves as a control procedure for the comparison of the effects of changing formula and technique in various ways.

Salt cannot be omitted from the formula because many of the doughs are too sticky for convenient manipulation during the test.

But salt can be reduced to 1%, which is the level used in baking tests and produces manageable doughs. An absorption identical with that used for baking is the most logical selection, and this corresponds in this laboratory to a consistency of 540 farinograph units. Since the basic test is not required to demonstrate the differential response of flours to mixing, a mild mixing procedure was selected. This is again identical with that used in the baking test, namely, 3 minutes in the Hobart mixer. Tests made with a wide range of flours have shown that the rest period of 45 minutes recommended by the Brabender Corporation is adequate for the relaxation of doughs. Accordingly, a 150-g. dough is scaled after mixing, is then rounded and rolled with the extensograph equipment, and is stretched after a 45-minute rest in a cabinet maintained at 86°F. and 85% relative humidity.

This technique is used mainly for comparative studies of varieties, especially for testing new lines and selections produced by plant breeders. When material is limited, useful results can also be obtained with a 75-g. dough without varying the technique in any other way. Curves are smaller, but their measurements are comparable to those of curves for 150-g. doughs.

Provided that procedure and formula do not vary widely from those described above, there will be little difference between the curves drawn 45 minutes after mixing. As a broad generalization, it may thus be said that all 45-minute curves represent essentially the innate properties of the flour. To measure the effect of treatments such as severe mixing or bromation, a second curve must be drawn. In this laboratory the second curve is prepared 135 or 180 minutes after mixing, with the proviso that the dough be rounded and rolled 45 minutes before stretching. The difference between the first curve (45 minutes) and the second curve (135 or 180 minutes) is considered to represent the response of the flour to treatment; for if the treatment were not varied from that of the basic procedure, the first and second curves would be almost identical. Perhaps there is here involved a mere question of terminology; and "innate properties" and "response" may not be the best terms available. Nevertheless, these terms do appear to convey a useful concept of what the extensograph measures and of how the results should be interpreted.

The principal limitation of the extensograph is that it does not measure specific physical properties such as elasticity and viscosity. The machine stretches a dough at constant rate and measures extension and resistance to extension. Though these properties depend upon the elasticity and viscosity of the dough, they are recorded in units that cannot be interpreted clearly in terms of either. The units have no independent meaning. They can be defined only in terms of the

machine, of its adjustment, and of the technique used in making the test. Accordingly, the units are wholly empirical, and this in itself sets a limit to the usefulness of the machine and of others like it. Ideal measurements of the rheological properties should yield fundamental constants such as elastic moduli and viscosity coefficients and should not depend on the machine used. Among dough studies of this type, those of Halton and Scott Blair (2, and papers cited therein) are preeminent. But their equipment and technique are hardly suitable for routine studies. Accordingly, the Brabender Extensograph, which is extremely convenient and simple to operate, will probably continue to be widely used in spite of its limitations. -

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INACTIVATION AND REMOVAL OF PROTEOLYTIC ENZYMES FROM AMYLOLYTIC SUPPLEMENTS¹

B. M. DIRKS² and BYRON S. MILLER³

ABSTRACT

Silicates proved superior to several other commercial adsorbents for selectively removing proteolytic enzymes from amylolytic supplements.

It was observed that 50 to 60% of the proteolytic activity in a fungal extract was inactivated rapidly and irreversibly by adjusting the pH to 10.0, with little decrease in amylolytic activity.

The addition of sodium chloride (4.3 *M*) to a fungal extract caused marked reduction of proteolytic activity and relatively little loss of amylolytic activity. Dialysis against 0.2% calcium chloride solution for 36 hours did not reactivate the proteolytic enzymes. In the presence of sodium chloride (4.3 *M*), adjustment of the extract to pH 10.0 resulted in 80 to 90% reduction of the original proteolytic activity with a small, but significant, loss of alpha-amylase.

Similar effects were noted in extracts of malted wheat flour and malted barley. The proteolytic activity of bacterial amylolytic preparations, however, was only slightly affected by increasing the reaction to pH 10.0. High concentrations of sodium chloride inactivated relatively large amounts of bacterial amylolytic, as well as proteolytic, enzymes.

Supplementation of flour with excessive amounts of alpha-amylase preparations has proved harmful in many instances. The reasons for these detrimental effects have been the subject of much research. Miller and Johnson (7) reviewed the literature dealing with this problem and presented data supporting the premise that the effects are due primarily to high proteolytic activity present in amylolytic supplements. Since controlled proteolytic activity is important in brewing and distilling processes as well as breadmaking, a study of methods for control of this enzyme activity in amylolytic preparations appears warranted.

Several general procedures have been used for purification and concentration of enzymes. These include dialysis, fractional precipitation, selective adsorption followed by elution, electrophoretic separation, and differential inactivation. The use of adsorbents has produced

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promising results, but the problem is complex and involves the interaction of many factors. The reaction or pH of the adsorbent suspension has a marked effect on the specificity and efficiency of the adsorption process. Lüers and Malsch (5) and Sabalitschka and Weidlich (8) found that malt amylase was best adsorbed at pH values approximating 4.5, while a pH optimum above 5.0 was observed for the adsorption of malt proteases. A series of investigations by Kitano (2, 3, 4) showed that the selectivity of any particular adsorbent for amylase or maltase was not absolute, but was influenced by temperature, pH, and enzyme concentration.

In 1938, Waldschmidt-Leitz and Ziegler (10) obtained a patent on the use of bauxite, a natural aluminum ore, for selectively adsorbing proteases from enzyme mixtures obtained from malted grains, molds, and bacteria. This process succeeded only in increasing the ratio of amylolytic to proteolytic enzymes. A pH value between 5.5 and 7.5 was recommended. Miller and Johnson (7) have investigated kaolin and found this agent also to be effective in differentially removing proteolytic enzymes although the separation was not complete. The purpose of the present work was to investigate various adsorbents and associated treatments in an effort to develop methods for the inactivation or removal of proteolytic from amylolytic enzymes.

Materials and Methods

The chief source of amylase investigated was that from the mold *Aspergillus oryzae*, cultured on wheat bran. Bacterial and cereal amylase preparations, as well as other fungal sources, were also used for comparative purposes. A solution of amylolytic and proteolytic enzymes was prepared by extracting the source with 0.2% calcium chloride solution at room temperature. A ratio of 1 g. of preparation to 10 ml. of 0.2% calcium chloride solution provided an extract of convenient enzyme activity. The mixture was stirred every 15 minutes for one hour after which it was filtered and centrifuged.

Commercial adsorbents used in this investigation were Duolite A-2, Duolite C-3, Florisil, Florex XXX, Isco-Adsorbol N-100, Isco-Adsorbol A-420, Santocel C, Santocel CX, Deacidite, Decalso Fines, Folin Decalso, Zeo-Dur, Zeo-Karb H, Zeo-Rex H, Amberlite IR4B, Amberlite IR100, and Zeolite. Other materials tested included Norit A, a decolorizing carbon of the Pfanstiehl Chemical Co., Waukegan, Ill.; Lloyd's Reagent, Hartmann-Leddon Co., Philadelphia; activated alumina; egg albumin; pumice; permutite; and Turkish emery.

Several additional adsorbents were prepared in the laboratory as follows:

Activated bauxite—

- A sample of pisolitic gray massive bauxite ore from Arkansas was finely ground and activated at 200°C. for 24 hours.

Hydrous aluminum oxide gel—

Prepared by adding 250 ml. of 3 *M* ammonium hydroxide solution to 250 ml. 0.5 *M* aluminum sulfate solution. The precipitate was washed, dried at 130°C., and activated for 24 hours at 200°C.

Hydrous aluminum oxide gel on asbestos support—

Prepared in the same manner as above but in the presence of 10 g. asbestos fiber.

Hydrous aluminum oxide gel in alcohol—

Prepared in the same manner as above with the alcohol being added at the time of adsorption.

Magnesium silicate—

Made by diluting sodium silicate to a specific gravity of 1.06 with distilled water and adding a molar solution of magnesium chloride to the silicate as long as precipitation occurred. The precipitate was washed, dried at 130°C., and activated 24 hours at 200°C.

Adsorption was carried out in a batch process at room temperature. A 25-ml. portion of the enzyme extract diluted 1:10 was added to 2 g. of the adsorbent in a 50-ml. beaker and stirred periodically during a 30-minute period. The pH of the suspension was measured immediately after mixing and pH adjustments were made at this time unless otherwise noted. After standing, the suspension was filtered with suction through Whatman No. 5 filter paper. Alpha-amylolytic activity and proteolytic activity were determined on appropriately diluted aliquots of the filtrate. All dilutions were made with 0.2% calcium chloride solution. This concentration (0.018 *M*) of calcium chloride did not inhibit proteolytic activity.

Proteolytic activity was determined by a modified Ayre-Anderson procedure as standardized by Miller (6). Briefly, this method involves the hydrolysis of a hemoglobin substrate by the enzyme extract in a mixture buffered at pH 4.7. After the hydrolysis period, undigested protein was precipitated with trichloroacetic acid, filtered off, and aliquots of the filtrate analyzed for total nitrogen by the Kjeldahl process. Titration values in ml. 0.0714 *N* sodium hydroxide indicated the proteolytic activity in the sample.

Alpha-amylase activity was determined by the starch dextrinization procedure described by Sandstedt, Kneen, and Blish (9). Values for alpha-amylase activity were expressed as the time required for the

digestion mixture to produce a standard red-brown end point with iodine.

Results and Discussion

Enzyme Concentration-Activity Relationships. Sandstedt, Kneen, and Blish (9) have established the linear relationship between alpha-amylase concentration and the rate of dextrinization, but no such simple relationship was evident in the determination of proteolytic activity. It was therefore necessary to determine the relation between proteolytic enzyme concentration and the titration values obtained from the activity determination. The activity expressed in ml. 0.0714 *N* sodium hydroxide (blank minus sample titrations) was plotted as shown in Fig. 1.

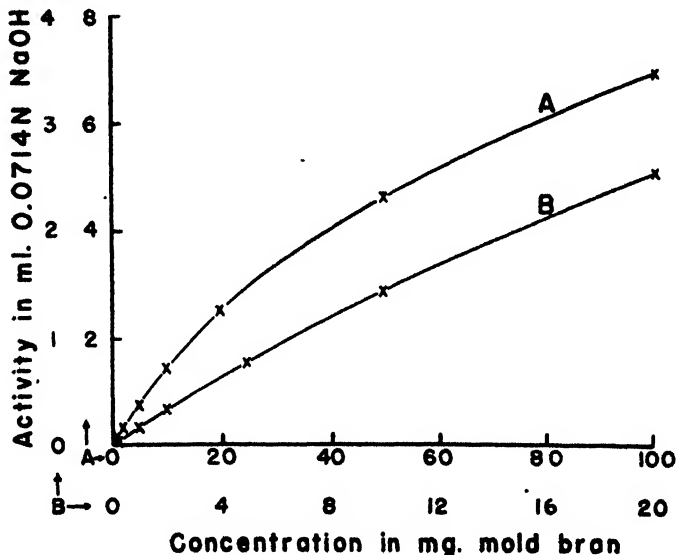


Fig. 1. Effect of concentration on measured proteolytic activity of mold bran extracts. Curve B represents the first portion of Curve A plotted on a different scale.

Curve A demonstrates the lack of proportionality between activity and enzyme concentration. Titration values consequently must be referred to a previously determined curve to get an accurate estimate of proteolytic activity. In this investigation it was convenient to dilute extracts so that titration values did not exceed 3 ml. These values fall on the portion of the curve plotted in curve B.

Examination of Adsorbents. Preliminary work was designed to eliminate those adsorbents showing little or no faculty for selective adsorption of proteolytic enzymes. Each adsorbent was tested in duplicate and the average results are given in Table I. These data

indicate that the use of Santocel C, Santocel CX, Zeo-Karb H, Zeo-Rex H, Isco-Adsorbol A-420, Duolite A-2, and Duolite C-3 resulted in pH levels that inactivated both proteolytic and amylolytic enzymes. These adsorbents should not be eliminated conclusively since preliminary treatment could adjust the pH to almost any level desired with probable improvement in selective properties. Other adsorbents including Florex XXX, Norit A, Zeo-Dur, egg albumin, and aluminum

TABLE I
EFFECTIVENESS OF VARIOUS ADSORBENTS IN SELECTIVE ADSORPTION
OF PROTEOLYTIC AND AMYLOLYTIC ENZYMES

Adsorbent	pH	Per cent loss in activity	
		Amylolytic	Proteolytic
Amberlite IR 4B	10.8	61.1	85.6
Amberlite IR 100	10.2	30.0	88.2
Florex XXX	6.5	92.7	97.6
Florisol	9.2	66.7	100.0
Santocel C	3.3	83.7	81.1
Santocel CX	3.5	85.0	80.4
Folin Decalso	6.3	7.2	33.8
Decalso Fines	7.2	7.2	61.8
Zeo-Dur	6.1	10.4	13.5
Zeo-Karb H	1.7	100.0	94.8
Zeo-Rex H	1.3	100.0	93.6
De-Acidite	7.6	29.0	51.8
Activated alumina	7.5	53.4	77.2
Magnesium silicate	10.2	22.8	78.9
Turkish emery	5.5	3.5	17.0
Pumice (pwd.)	5.8	13.0	44.4
Egg albumin	6.2	10.0	20.5
Isco-Adsorbol A-420	3.4	100.0	100.0
Isco-Adsorbol N-100	6.4	29.0	84.7
Al(OH) ₃	6.5	44.0	31.1
Al(OH) ₃ on asbestos	6.5	42.8	52.8
Al(OH) ₃ in alcohol	4.3	19.4	22.8
Activated bauxite	6.2	22.2	40.5
Zeolite	4.7	17.6	51.8
Norit A	7.2	100.0	97.1
Permutite	6.9	6.6	31.5
Duolite A-2	3.6	100.0	53.5
Duolite C-3	2.1	100.0	96.5
Lloyd's Reagent	6.7	12.1	78.0

hydroxide exhibited little selectivity in their adsorption characteristics under the conditions of these experiments.

The remaining adsorbents were subjected to further study by varying the pH through the addition of dilute hydrochloric acid or sodium hydroxide to the suspension immediately after mixing the enzyme extract and adsorbent. Figs. 2 and 3 show the results obtained with the eight adsorbents exhibiting the greatest relative preference for proteolytic enzymes.

Percentage losses of proteolytic and amylolytic activity are plotted against various pH levels for each adsorbent. Points represent average values of several determinations within a pH range of 0.5 unit. The degree of adsorptive selectivity for the adsorbent concentrations used is thus represented by the distance between the two curves at any particular pH value. Since higher values invariably resulted in increased losses of amylolytic activity, the curves were not extended far above pH 10.0. Adsorption treatment of extracts previously

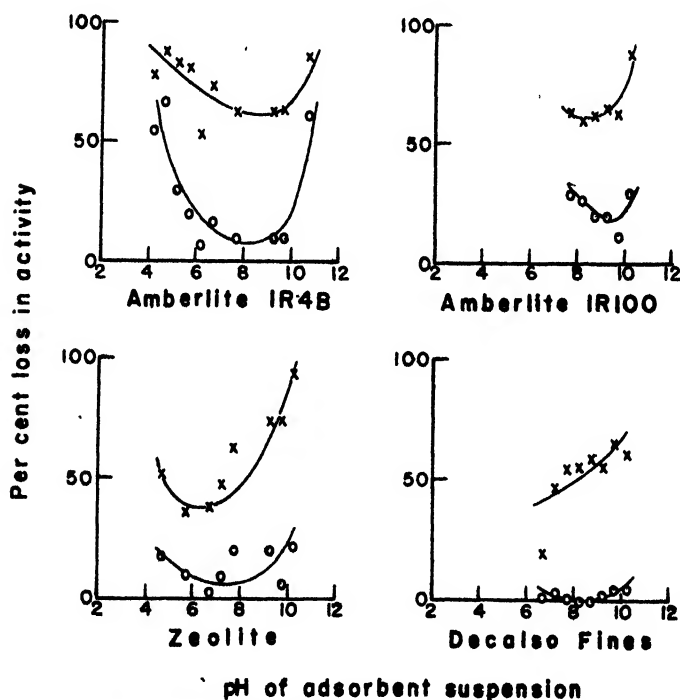


Fig. 2. Relationship of adsorption of amylolytic and proteolytic enzymes in mold bran extracts to changes in hydrogen-ion concentration for different adsorbents. Amylolytic activity is represented by "o" and proteolytic activity by "x."

adjusted to pH 10.0 decreased the proteolytic activity over 80% at the expense, however, of significant losses in amylolytic activity.

Removal of proteolytic enzymes from amylolytic supplements by adsorption was only partially successful. The adsorbents exhibiting the best selective properties were either silicates or were high in silicate content. Best results were obtained with Decalso, a synthetic sodium aluminosilicate; Lloyd's reagent, a form of hydrated aluminum silicate; and Isco-Adsorbol N-100, a natural clay. Additional work employing various quantities of specific adsorbents was abandoned

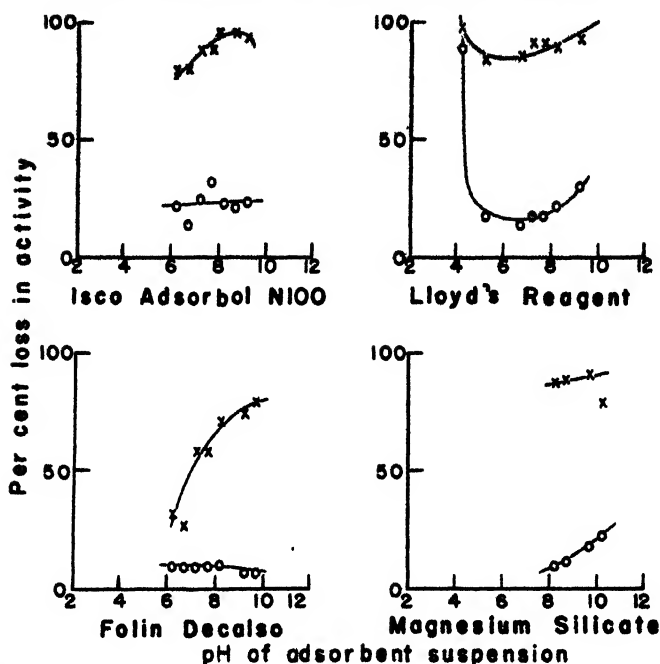


Fig. 3. Relationship of adsorption of amylolytic and proteolytic enzymes in mold bran extracts to changes in hydrogen-ion concentration for different adsorbents. Amylolytic activity is represented by "o" and proteolytic activity by "x."

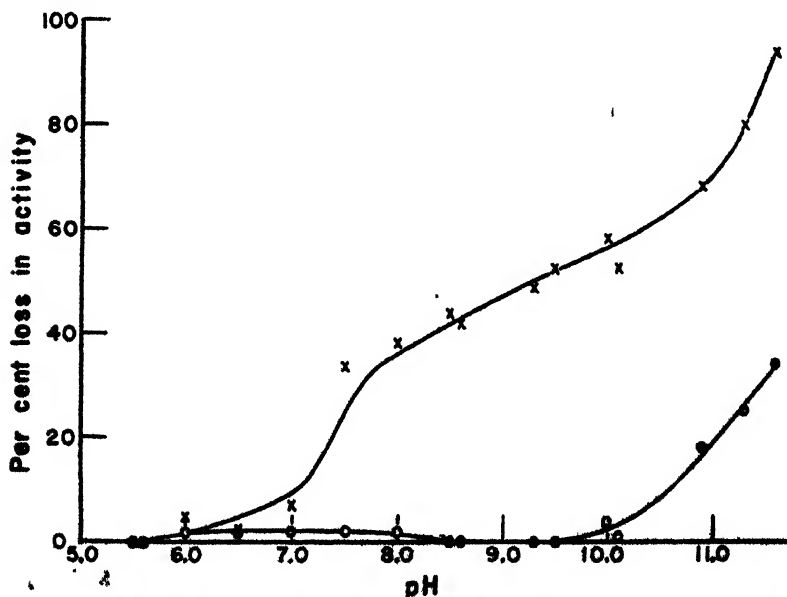


Fig. 4. Effect of hydrogen-ion concentration on the activity of amylolytic and proteolytic enzymes in mold bran extracts. Amylolytic activity is represented by "o" and proteolytic activity by "x."

due to the more promising results obtained by controlled hydrogen-ion and chloride-ion concentration.

Effect of Hydrogen-Ion Concentration. The curves in Figs. 2 and 3 show that the highest degree of adsorptive selectivity apparently occurred between pH 8.0 and pH 10.0 in nearly all cases. This fact stimulated further investigation of pH effects. The data plotted in Fig. 4 show that the effect of high pH values was largely due to selective inactivation rather than to adsorption. A pH of 10.0 resulted in the inactivation of over 50% of the proteolytic activity with no significant loss in amylolytic activity. Thus there appeared to be no advantage in the use of adsorbents for removal of proteolytic enzymes unless a selective adsorption of at least 50% over the amylolytic enzymes was obtained.

TABLE II

EFFECT OF HYDROGEN-ION ADJUSTMENT WITH DIFFERENT REAGENTS ON AMYLOLYTIC AND PROTEOLYTIC ACTIVITY OF MOLD BRAN EXTRACTS

Treatment	pH of extract	Per cent loss in activity	
		Amylolytic	Proteolytic
NaOH	10.1	1.9	51.7
Ba(OH) ₂	10.0	5.9	45.5
NaOH + HCl	5.6	12.7	61.4
NaOH + HAc	4.6	5.8	53.3

Table II reveals that adjustment of pH with dilute barium hydroxide achieved the same degree of inactivation as adjustment with dilute sodium hydroxide. The resulting proteolytic activity after 3 hours was virtually the same as when measured immediately after pH adjustment. Further the proteolytic enzymes did not recover their activity either with standing as long as 36 hours or with subsequent lowering of the pH level. The inactivation, apparently due to hydroxyl ions, was concluded to be irreversible and very rapid. Re-adjustment of pH by the addition of dilute hydrochloric acid caused an additional loss of proteolytic activity. Since acetic acid caused no significant changes, it was assumed that the additional loss of proteolytic activity with the addition of hydrochloric acid was due to the chloride ion.

Effect of Sodium Chloride. Table III shows the effect of sodium chloride on enzyme activity when the salt was added to the hemoglobin digestion mixture, the enzyme extract, and the extraction slurry. A salt concentration equivalent to 4.3 M in the extract reduced the proteolytic activity about 20 to 30% with little loss of amylolytic activity irrespective of the time of addition. As noted previously, the

TABLE III
EFFECT OF SODIUM CHLORIDE ON AMYLOLYTIC AND PROTEOLYTIC
ACTIVITY OF MOLD BRAN EXTRACTS

Treatment	NaCl molarity	pH of extract	Per cent loss in activity	
			Amylolytic	Proteolytic
In digest	0.6 ¹	5.2	14.8	66.4
In digest	0.2 ²	5.2	2.0	26.2
In extract	0.5	5.2	0.0	3.3
In extract	4.3	4.8	5.8	25.2
In slurry	4.3	5.4	3.8	17.6

¹ 0.6 M sodium chloride in the digestion mixture represents the same concentration as that usually mixed in bread doughs.

² 0.2 M sodium chloride in the digestion mixture is equivalent to 4.3 M sodium chloride in the enzyme extract.

original mold bran source was extracted with 0.2% calcium chloride solution. This represents only 0.036 M chloride ion, and at least 0.5 M chloride ion was necessary before any appreciable loss of proteolytic activity was detected. Since concentrations of calcium chloride supplying equivalent amounts of chloride ion achieved results similar to those obtained with sodium chloride, the results were apparently due to the chloride ion present.

Dialysis of treated extracts for 36 hours at 30°C. against 0.2% calcium chloride solution resulted in no change in activity. Quantitative determinations of chloride ions showed the chloride concentration to be reduced to 0.036 M during this period. Treated and untreated check samples allowed to stand for the same length of time at 30°C. exhibited no change in activity from that measured immediately after treatment. The inactivating influence of chloride ions consequently appears to be quite rapid and irreversible.

Combined Effect of Hydrogen-ion Concentration and Sodium Chloride. The additive effect of hydroxyl and chloride ions on proteolytic

TABLE IV
COMBINED EFFECT OF SODIUM CHLORIDE AND HYDROGEN-ION CONCENTRATION ON
THE AMYLOLYTIC AND PROTEOLYTIC ACTIVITY OF MOLD BRAN EXTRACTS

Treatment	NaCl molarity	pH of extract	Per cent loss in activity	
			Amylolytic	Proteolytic
NaOH	—	10.1	1.9	51.7
NaCl	4.3	4.8	5.8	25.2
NaCl+NaOH	4.3	10.1	6.5	86.2
NaCl+NaOH (in slurry)	4.3	6.9 ¹	10.7	95.1

¹ The slurry was adjusted to pH 10.0 before centrifuging and diluting for analysis.

activity is shown in Table IV. Treatment of mold bran extracts at pH 10.0 with 250 mg. sodium chloride per ml. resulted in over 80% inactivation of proteolytic enzymes and less than 10% loss of amylolytic enzymes. Significant losses of amylolytic activity prevented the use of higher concentrations of either hydroxyl or chloride ions. Additional work concerning the effect of other ions is in progress.

Comparison of Enzyme Sources. Table V shows the effects of hydroxyl and chloride ions on the enzyme activity of amylase preparations from various sources. Proteolytic and amylolytic enzymes from several fungal sources reacted similarly to treatment with sodium chloride and sodium hydroxide. The cereal and vegetable enzymes also were inactivated in the same manner, although the proteolytic

TABLE V

EFFECT OF HYDROGEN-ION CONCENTRATION AND SODIUM CHLORIDE ON THE AMYLOLYTIC AND PROTEOLYTIC ACTIVITY IN EXTRACTS OF DIFFERENT ENZYME SOURCES

Source	Type	Per cent loss in activity					
		pH 10.0		0.6 M NaCl ¹		pH 10.0, 0.6 M NaCl	
		Amyl.	Prot.	Amyl.	Prot.	Amyl.	Prot.
Goering bran	Fungal	0.0	52.0	14.8	66.4	14.8	88.0
Wallerstein bran	Fungal	4.8	76.5	9.1	54.1	16.7	88.8
E-Z-1	Fungal	0.0	75.0	10.5	48.3	10.5	86.7
Rhozyme S	Fungal	0.0	85.7	5.9	45.7	5.9	92.1
Wallerstein	Bacterial	0.0	1.1	10.5	40.4	10.5	40.4
Jeffrey's bran	Bacterial	3.8	3.9	35.1	71.0	35.9	80.6
Polidase-S	Vegetable	0.0	30.5	0.0	56.6	5.3	78.6
Malted barley	Cereal	0.0	70.6	2.4	26.5	4.7	78.4
Malted wheat	Cereal	0.0	30.4	6.7	15.8	12.5	42.9

¹ 0.6 M sodium chloride in the digestion mixture represents the same concentration as that usually mixed in bread doughs.

activity of malted wheat flour was not decreased to the same extent as in malted barley and fungal extracts. For all fungal and cereal sources tested, there was a definite additive effect of pH and salt inactivation resulting in as much as 90% loss of proteolytic activity with as little as 6% loss of amylolytic activity.

Bacterial sources, however, provided proteolytic enzymes with different properties. Proteolytic activity was not decreased significantly at pH 10.0, but amylolytic activity decreased sharply with pH values above 10.0 in the same manner as the fungal amylolytic activity. The same inactivation pH for bacterial amylases was reported by Di Carlo and Redfern (1). Treatment with sodium chloride achieved a degree of proteolytic enzyme inactivation comparable to that of fungal sources. With higher salt concentrations it was possible to

obtain an inactivation of bacterial proteolytic activity roughly equivalent to that obtained with fungal extracts treated with both salt and base. However, a relatively large amount of amylolytic activity also was lost.

The inactivation or removal of proteolytic enzymes from amylolytic supplements was not complete, but a significant increase in the ratio of amylolytic to proteolytic activity was successfully obtained. However, only a few of the many aspects of the problem have been covered.

The use of adsorption and elution techniques should be exploited to determine proper conditions for this separation. Investigations of the amylolytic and proteolytic enzyme systems of cereals, molds, and bacteria would then be facilitated.

Acknowledgments

Acknowledgment is made to the several commercial concerns who generously supplied the adsorbents and enzyme preparations used in this investigation. The mold bran used as the chief source of amylase studied was obtained through the courtesy of Dr. K. J. Goering, President, the Mold Bran Company, Eagle Grove, Iowa. Additional fungal sources included "E-Z-1" from the Mold Bran Company; a mold bran product of the Wallerstein Laboratories, New York; and Rhozyme-S, Rohm and Haas Company, Philadelphia. A bacterial bran of the Jeffreys Laboratories, Salem, Virginia, was used as well as a Wallerstein bacterial amylase preparation. Also examined were a commercial malted wheat flour, the Kansas Milling Company, Wichita, Kansas; a commercial malted barley, Hales and Hunter Company, Chicago; and Polidase-S, a vegetable amylase preparation of the Schwartz Laboratories, Inc., New York.

Samples of adsorbents were obtained from the following sources:

The Chemical Process Co., 58 Sutter Street, San Francisco—Duolite A-2, Duolite C-3.

The Floridin Co., Warren, Pennsylvania—Florisol, Florex XXX.

Innis, Spieden and Co., 117 Liberty St., New York—Isco-Adsorbol N-100, Isco-Adsorbol A-420.

Monsanto Chemical Co., Merrimac Div., Boston—Santocel C, Santocel CX.

The Permutit Co., 330 West 42nd St., New York—Deacidite, Decalso Fines, Folin Decalso, Zeo-Dur, Zeo-Karb H, Zeo-Rex H.

The Resinous Products and Chemical Co., Washington Square, Philadelphia—Amberlite IR4B, Amberlite IR100.

Zeolite Chemical Co., 140 Cedar St., New York—Zeolite.

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GRAIN STORAGE STUDIES. VIII. RELATION OF MOLDS IN MOIST STORED COTTONSEED TO INCREASED PRODUCTION OF CARBON DIOXIDE, FATTY ACIDS, AND HEAT¹

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ABSTRACT

Storage molds increased rapidly on and within cottonseed stored at relative humidities of 84% to 90% for 10 days at 30°C. In general, the increase in mold population of the stored seeds was correlated closely with increased production of carbon dioxide and free fatty acids. Because of the peculiar structure of the seed coat, molds gain easy access to the interior of moist stored cottonseed where they grow vigorously and sporulate profusely, so that cottonseed may be extremely moldy without betraying any external evidence. Fungicides applied to the outside of the seed did not prevent rapid growth and sporulation of molds within the seed coat. Entire meats and hulls stored moist without a fungicide respired more rapidly, had a higher fatty acid content, and bore a larger mold population than similar fractions treated with thiourea. The evidence suggests that storage molds may play an important part in the deterioration of moist stored cottonseed.

During the past 20 years considerable evidence has accumulated to indicate that molds often are a major factor in the spoilage and heating of moist stored seeds of various kinds (8, 16-23). Whether molds are similarly involved in the deterioration of moist stored cottonseed has been questioned, although cottonseed is known to bear a fairly rich internal flora of both parasitic and saprophytic molds (3). Malowan (15) found that the respiration of moist cottonseed was not reduced when the seed was treated with copper sulfate or mercuric chloride,

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and concluded that therefore microorganisms were not involved in its respiration. He did not determine whether the mold population of the seeds increased or decreased during his tests, and therefore had no experimental basis for supposing that the seed treatments he used eliminated molds. Darsie *et al.* (6), some years before, had found that treatment of seeds with copper sulfate did not prevent subsequent molding and heating of the seeds, and mercuric chloride was not always completely effective in eliminating molds: That fungicides applied to seeds may not inhibit the subsequent development of storage molds on the seed was shown by Milner, Christensen, and Geddes (17). In the absence of direct supporting evidence, therefore, Malowan's conclusion that microflora were not concerned with respiration of the seeds in his tests must be regarded as purely hypothetical. More recently, workers at the Southern Regional Research Laboratory have expressed some doubt as to whether molds are involved in the deterioration of moist stored cottonseed (1, 11, 12, 14). They favor the view that the major lipolytic, respiratory, and heating activity of cottonseed in storage is due to seed enzymes and that microbiological activity is, at best, a secondary factor which only comes into play after long storage. However, they did not determine the mold population in their experiments; and whether molds are involved in the deterioration of moist stored cottonseed, as they have been proven to be with other kinds of seed, remains a moot question. The present work was undertaken to explore some aspects of this problem.

Materials and Methods

The cottonseed used in these tests was, unless otherwise stated, nondelinted seed of the variety Stoneville, obtained from the Southern Regional Research Laboratory at New Orleans, Louisiana. In the various tests it was conditioned to the desired moisture contents by adding distilled water, then storing the seed at 2°–4°C. for 36 to 48 hours to allow the water to become distributed. Moisture content was determined by the method specified for cottonseed by the American Oil Chemists' Society (2). Carbon dioxide production was measured by the technique described by Milner and Geddes (18); the experimental lots of seed at various moisture contents were kept in respirometer bottles at 30°C., and air in hygroscopic equilibrium with the initial moisture content of the seed was continuously passed through the seed. At intervals of 24 hours the accumulated air was analyzed for carbon dioxide and oxygen, using a modified Haldane-Henderson gas analyzer.

Fat acidity was measured by a slight modification of the A.O.C.S. method for cottonseed, (2). The intact seed was delinted with con-

concentrated sulfuric acid, thoroughly washed in a stream of water, and air dried. The material was ground in an intermediate Wiley mill equipped with a 20 mesh screen. Samples of 20 g. were extracted at room temperature by allowing 100 ml. of petroleum ether to percolate through the material in a Butt-type extraction tube. The extracts were collected in weighed flasks and were reweighed, after evaporation of the solvent, to give the weight of oil present in the titration mixture. Subsequent treatment followed that recommended by the American Oil Chemists' Society.

The mold population was determined by grinding the seed in a small attrition mill turned by hand or in a Wiley mill equipped with a 20 mesh screen, and culturing the resultant meal in malt-salt agar as described by Christensen (4). It was difficult to reduce these oily seeds to a fine meal in the attrition mill, but eventually it was found that if the seeds were dried thoroughly, then chilled or frozen, they could be ground satisfactorily in the Wiley mill. Because of the unsatisfactory milling of some seed lots, it is likely that mold counts on these lots were lower than the actual population, but this probably had relatively little effect on the general results. To determine the percentage of seeds in which internal molds were present, 50 seeds were delinted in concentrated sulfuric acid, washed in running tap water, then surface-disinfected by washing in a 1.5% solution of sodium hypochlorite for 2 minutes (to eliminate any contaminants acquired from the tap water or from the air), placed on agar in petri dishes, and notes taken after 5 to 7 days.

The percentage germination of the seed was determined by the Minnesota State Seed Testing Laboratory.

Results

Relation of Moisture Content to Respiration, Mold Population, and Increase in Fatty Acids. Portions of seed weighing from 250 to 600 g. were conditioned to six different moisture contents, placed in respirometer bottles, and aspirated with air in hygroscopic equilibrium with each moisture content. In no case did the final moisture content differ from the initial by more than 0.5%. After storage for 10 days, the seeds were removed, dried, and the fat acidity, germination, total mold population, and percentage of seed with internal molds determined. The results are presented in Table I. The increasing internal infection by storage molds with increasing moisture content is illustrated in Fig. 1.

Respiration, fat acidity, and mold population increased slightly during the 10-day storage period at 80% relative humidity, and increased greatly at relative humidities of 84 to 90%. At 90% relative

TABLE I

CARBON DIOXIDE PRODUCTION, FAT ACIDITY, AND MOLD POPULATION IN COTTONSEED STORED AT SIX DIFFERENT RELATIVE HUMIDITIES, AT 30°C., FOR 10 DAYS

Number	Relative humidity	Moisture content	Respiration ¹	Fat acidity		Germination	Molds		Kinds of molds
				As oleic	Acid value ²		Total number ³	Internal infection ⁴	
Original seed	%	%	mg.	%	mg.	%		%	
1	65.0	9.6	5.6	0.4	8.1	97	700	6	<i>Fusarium vasinfectum</i>
2	75.0	11.6	7.0	0.5	10.4	93	0	8	<i>Fusarium vasinfectum</i>
3	80.0	13.4	21.1	0.6	11.9	88	700	0	Not identified
4	84.0	15.2	79.0	0.9	16.8	91	1,000	6	<i>Aspergillus glaucus</i>
5	87.0	17.4	406.2	1.3	26.0	90	3,300	24	<i>A. glaucus</i> and <i>A. versicolor</i>
6	90.0	19.8	1069.2	6.3	125.1	66	152,000	24	<i>A. glaucus</i> and <i>A. versicolor</i>
				9.4	186.6	55	506,000	66	<i>A. glaucus</i> and <i>Penicillium</i> sp.

¹ Total mg. CO₂/100 g. dry matter in 10 days.

² Expressed as mg. KOH/10 g. of oil.

³ Molds per gram.

⁴ Percentage of seeds with internal molds.

humidity, the total carbon dioxide production was over twice as great as at 87%, free fatty acids about one and one-half times as great (about six times greater than at 84% relative humidity), the mold population about three times as great, nearly half the seeds were dead, and more than half had been invaded by storage molds.

Over the 10-day period, the daily carbon dioxide values were low, constant, and nearly equal for the samples containing 9.6 and 11.6%

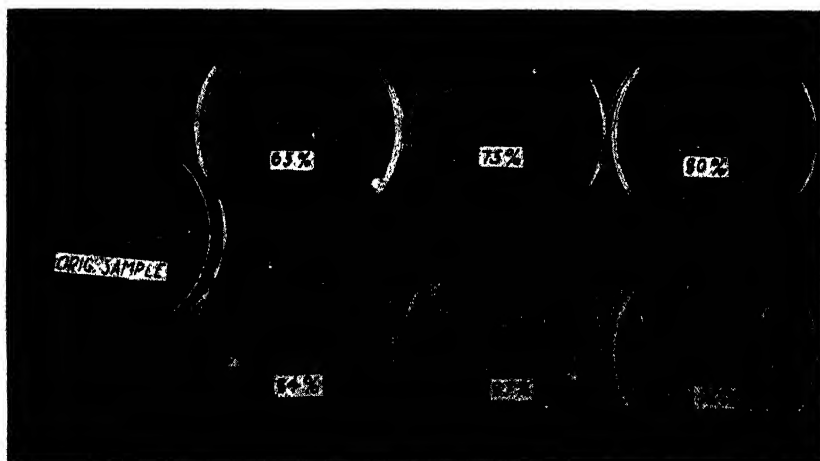


Fig. 1. Cottonseed stored at the indicated relative humidities for 10 days, then delinted, surface-disinfested and placed on malt-salt agar. Storage molds increased rapidly above 84% relative humidity.

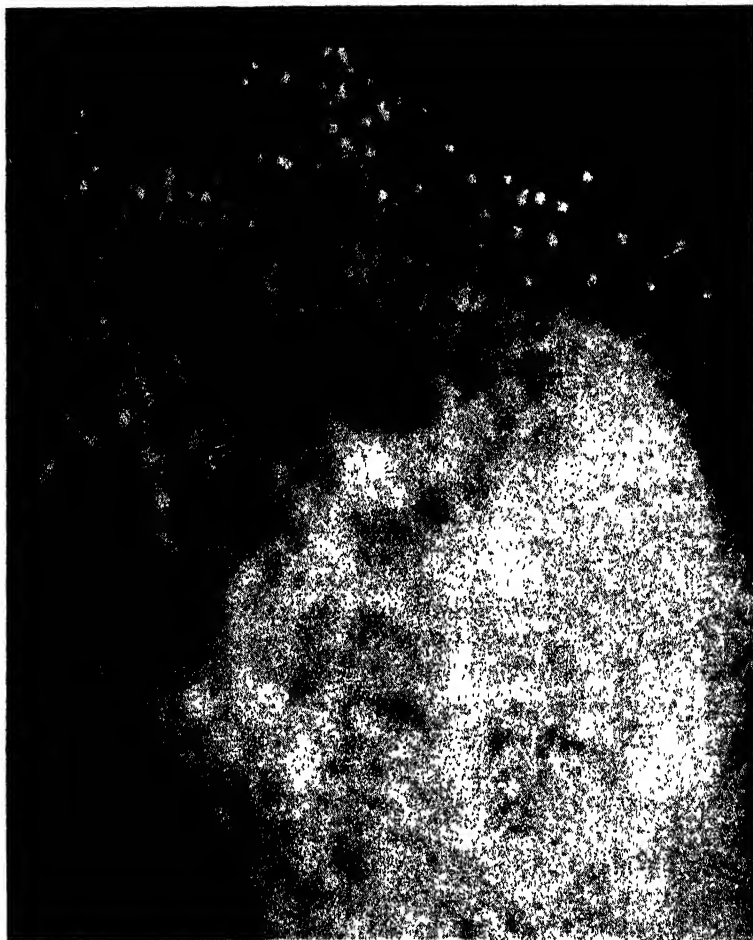


Fig. 2. *Aspergillus ochraceus* growing from basal end of cottonseed stored 10 days at 90% relative humidity. The original seed had less than 1,000 molds per gram, and none of the seed was internally infected with storage molds. After 10 days at 90% relative humidity, the seed had about 350,000 molds per gram and typical storage molds were present internally in more than 50% of the individual seeds.

moisture. The daily values for the samples at 13.4 and 15.2% moisture were somewhat higher and increased slightly with time. The samples maintained at 17.4% and 19.7% moisture had somewhat higher initial respiration rates than those at lower moistures and the daily rates markedly increased as the trial progressed to give carbon dioxide-production rate curves similar in shape to a microbiological growth curve.

The samples which respired at 9.6, 11.6, 13.4, and 15.2% moisture exhibited respiratory quotients of 0.6 to 0.7, whereas the daily respiratory quotients of the samples maintained at 17.4 and 19.7% moisture

increased with time and approached 1.0 at the end of the trial. This tendency of the respiratory quotients to increase when conditions are favorable for mold growth has been observed in similar experiments with soybeans and wheat (16, 19). The increasing respiration, increasing fat acidity, and decreasing viability associated with mold increase were essentially the same as have been found in similar tests (16, 18, 19) with other types of seeds stored under similar conditions. The species of molds that invaded the seeds were also essentially the same as in previous studies with other grains.

Mold sporophores were easily visible to the naked eye on seeds that had been stored at 90% relative humidity for only 10 days, as shown in Fig. 2. As will be described later, the structure of cottonseed enables molds to gain easy access to the interior of the seed, and the principal mold development is often *within* the seed coat, not outside, so that mere visual examination of the seed is an unreliable criterion of moldiness. However, in the test just described, mold sporophores were numerous and obvious to the naked eye on some of the seeds stored at 90% relative humidity.

Effect of Differential Aeration on Respiration, Fat Acidity, and Mold Population. Nine hundred grams of nondelinted seed were conditioned to 19% moisture and divided into six subsamples. Five of these were aerated at 30°C. for 13 days with 250 to 2,000 ml. of air per 24 hours, while the sixth subsample received 250 ml. of nitrogen (tank nitrogen containing approximately 0.3% oxygen). The relative humidity of the gases passed through the seed was maintained at 90%; at the end of 13 days' storage, the moisture content of the six subsamples varied from 19.1 to 19.6%. Carbon dioxide production, free fatty acids, total mold population, percentage of seeds with internal molds, and seed viability at the end of the 13-day storage period are presented in Table II and typical internal molds are illustrated in Fig. 3.

The daily respiratory rates for the sample which received nitrogen (containing about 0.3% oxygen) were practically constant throughout the 13-day trial. As the effluent nitrogen contained from 4.4 to 4.8% carbon dioxide, some anaerobic respiration occurred. The samples which received 250 and 500 ml. of air per day showed increased respiratory rates until the sixth and seventh days after which the respective rates were nearly constant. Between the sixth and thirteenth days the effluent air from the sample aerated at 250 ml. per day contained approximately 19.0% of carbon dioxide and that from the sample aerated at 500 ml. contained from 14.5 to 16.7% of carbon dioxide. The samples aerated with 1,000, 1,500, and 2,000 ml. of air per day gave increasing respiratory rates throughout the entire 13-day trial, although the rates for the sample which received 1,000 ml. of air per

day showed a tendency to level off during the last day of the trial. At this time, the effluent air contained 13.4% of carbon dioxide. The respiratory rates for the three samples receiving the highest aerations accelerated in a very similar manner until the sixth day when rate of acceleration for the sample receiving 1,000 ml. per day began to lag behind the other two. The effluent air from this sample contained 8.5% of carbon dioxide on the sixth day and 13.4% on the thirteenth day, and these carbon dioxide levels therefore partially inhibit respiration. The carbon dioxide rate curves for the samples aerated with 1,500 and 2,000 ml. per day were parallel and accelerated through the entire trial, although the rate of acceleration somewhat decreased

TABLE II

EFFECT OF DIFFERENTIAL AERATION ON RESPIRATION, FAT ACIDITY,
AND MOLD POPULATION IN COTTONSEED STORED AT 19%
MOISTURE AND 30°C., FOR 13 DAYS

Number	Aeration		Respi- ration ¹	Fat acidity		Germi- nation	Molds		Kinds of molds
				As oleic	Acid value ²		Total number ³	Internal infection ⁴	
	Gas	ml.	mg.	%	mg.	%		%	
Original seed	—	—	—	0.4	7.8	97	1,000	6	
1	Air	2000	2186.9	12.2	242.2	41	1,023,000	100	<i>Aspergillus glaucus</i> chiefly
2	Air	1500	2246.5	11.0	219.1	40	660,000	92	<i>Aspergillus glaucus</i> chiefly
3	Air	1000	1858.2	9.1	181.0	47	230,000	100	<i>Aspergillus glaucus</i> chiefly
4	Air	500	1398.1	4.8	95.2	66	240,000	100	<i>Aspergillus glaucus</i> chiefly
5	Air	250	838.3	2.7	53.0	81	—	98	<i>Aspergillus glaucus</i> chiefly
6	Nitro- gen	250	198.8	0.5	9.1	44	<1,000	46	Probably <i>Glomerella gos- sypii</i>

¹ Total mg. CO₂/100 g. dry matter in 13 days.

² Expressed as mg. KOH/10 g. of oil.

³ Molds per gram.

⁴ Percentage of seeds with internal molds.

after the eleventh day. Under the conditions of this experiment 1,500 ml. of air per day were adequate to obtain maximum respiration of 150 g. of cottonseed. The results indicate that carbon dioxide concentrations above about 8.5% exert an inhibiting effect upon carbon dioxide production.

The mold population decreased somewhat with decreasing aeration, although even 500 ml. of air per 24 hours were sufficient to permit considerable increase in both molds and free fatty acids. In the sample which received nitrogen, the respiration was low, fatty acids scarcely increased, and no typical storage molds developed, but at the end of the test nearly 50% of the seeds were infected with an unidentified white mold. *Glomerella gossypii* and *Fusarium vasinfectum* were

later found to be present in many of the seeds used in these tests. These fungi are common seed-borne parasites of cotton (3), and the fungus which grew from the seeds stored under nitrogen resembled, in gross cultural characteristics on malt-salt agar, that which was later positively identified as *G. gossypii*.

In this test, some of the seed that bore more than 200,000 molds per gram could not be distinguished, by external examination with the naked eye, from the original seed that had fewer than 1,000 molds per gram. At the risk of belaboring an obvious point, it might be stated

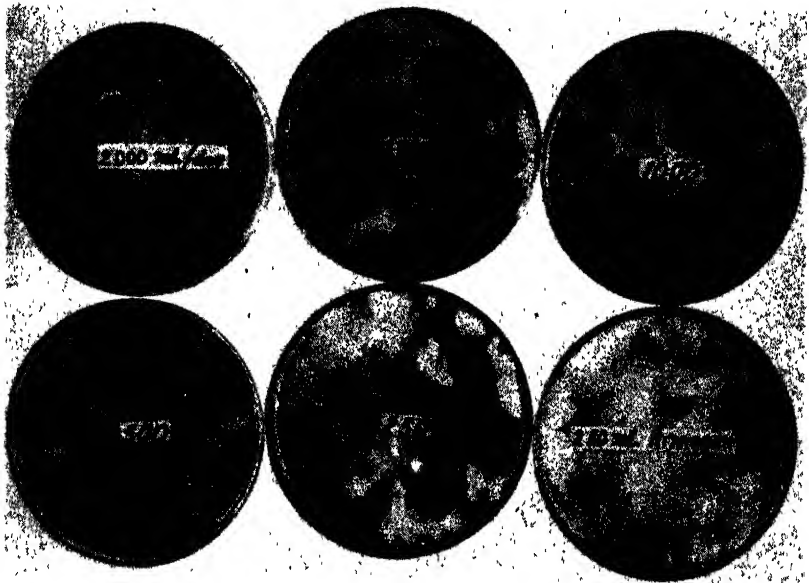


Fig. 3. Cottonseed stored at 90% relative humidity for 13 days, aspirated with the amounts of air (or nitrogen) in ml. per day indicated by the labels. After 13 days the seeds were delinted, surface-disinfected, and placed on agar. Typical storage molds were present in all seeds except those which received 250 ml. of air or nitrogen. No internal storage molds were found in the latter.

that this is additional evidence that relative moldiness of the seed cannot be accurately determined by visual examination.

Respiration, Temperature Increase, and Mold Development in Adiabatic Respirometer, with and without Thiourea as a Mold Inhibitor. Nondelinted seed was conditioned to 18% moisture, replicate lots of 100 g. each were weighed out, and to half of these 1.0 g. of finely powdered thiourea per 100 g. of dry seed was added to inhibit molds. Thiourea was chosen because, in tests of more than 100 compounds by Milner, Christensen, and Geddes (17), it effectively inhibited molds on wheat at a comparable moisture content, and did not greatly reduce viability of the seed. The samples were aerated for 23 days in the

adiabatic respirometer described by Milner and Geddes (16) at the rate of 2,000 ml. of air per 24 hours. Carbon dioxide production and temperature were measured daily and, at various intervals, samples were removed for determinations of mold population and fat acidity. The results are presented in Table III.

The nontreated seed heated more rapidly than that treated with thiourea, and, in both, the mold increase was roughly proportional to the temperature rise. The nontreated sample attained a temperature of 37°C. in 7 days, and then had a mold population of 470,000 per gram, while the seed treated with thiourea only attained a temperature of 33.1°C. in 16 days, and then bore 450,000 molds per gram. After 9

TABLE III

CARBON DIOXIDE PRODUCTION, TEMPERATURE INCREASE,¹ AND MOLD POPULATION OF COTTONSEED STORED IN ADIABATIC RESPIROMETER WITH AND WITHOUT THIOUREA AS A MOLD INHIBITOR¹

Treatment	Days stored	Respiration ²	Temperature	Fat acidity		Molds per g
				As oleic	Acid value ³	
		mg.	°C.	%	mg.	
Untreated	7	163.9	37.0	1.2	23.9	470,000
	9	335.7	45.9	2.1	42.5	287,000
	16	77.1	65.7	2.6	52.7	0
	20	78.9	68.8	—	—	—
	23	99.3	74.8	2.9	57.0	—
Thiourea	7	21.4	26.2	—	—	—
	9	24.3	27.0	—	—	—
	16	68.9	33.1	0.4	8.5	450,000
	20	219.0	46.5	0.8	16.3	300,000
	23	88.9	57.0	1.3	26.8	0

¹ The original seed had 100 molds per gram and a free fatty acid content of 0.4% expressed as oleic acid equivalent to an acid value of 7.8.

² Total mg. CO₂/100 g. dry matter.

³ Expressed as mg. KOH/10 g. of oil.

days' storage, the nontreated seed had a temperature of 45.9°C. and a mold population of 287,000 per gram, while the seed treated with thiourea required 20 days to attain a temperature of 46.5°C. and a mold population of 300,000 per gram. The chief mold present in both samples was *Aspergillus glaucus*. Previous work in these laboratories has shown that this mold gradually is eliminated after the temperature rises above approximately 40°C., and so the reduction in mold population as the temperature exceeded 45°C. was not unexpected. No molds were found on the seed after the temperature exceeded 55°C., as was to be expected, since none of the molds encountered on these seeds will survive above that temperature.

The preliminary stages of heating of the moist seed were accompanied by vigorous mold growth of the same species as has been found to accompany preliminary heating in other grains (16, 22, 23). Once the temperature exceeded 45°C. the heating process was faster than has been found in soybeans or wheat; and molds such as *Aspergillus flavus*, *A. candidus*, *A. fumigatus*, and *Mucor* sp., which grow vigorously on the latter seeds between 45° and 50°C., were not encountered on cottonseed in the present test.

That powdered thiourea, in the concentration used, failed to prevent mold growth and heating was easily explained once the seeds were sectioned and examined internally. There was little or no external mold on the seed treated with thiourea, but, after two weeks' storage,

TABLE IV

INFLUENCE OF FIVE FUNGICIDES, APPLIED AS DUSTS IN A CONCENTRATION OF 1:100, ON THE INCREASE OF MOLDS IN COTTONSEED STORED AT 19.0% MOISTURE¹

Fungicide	Seeds with internal molds after 30 days	Internal molds per gram		Principal kinds of molds
		Storage time		
		30 days	60 days	
Control	%	11,000	800,000	Mixed flora
Arasan	26	210,000	13,000,000	Penicillium
Thiourea	92	1,500,000	14,000,000	Penicillium
Spergon	70	3,200,000	22,000,000	Penicillium
Chloramine-B	38	—	36,000	<i>Aspergillus candidus</i>
8-hydroxyquinoline	8	—	100,000 ²	<i>A. glaucus</i>

¹ The original seed had less than 1000 molds per gram and 18% were infected with internal molds.

² All of these colonies arose from particles of the seed coat, indicating that the seed coat itself had been invaded by mold mycelium. The colonies grew slowly, and apparently were inhibited by the fungicide remaining in the seed coat, or by that which had diffused from the seed coat into the agar medium.

many of the seeds had a heavy internal mold flora which could be seen readily with a binocular microscope in sectioned seeds. This suggests that external application of a compound known to be fairly toxic to most storage molds will not necessarily protect the seed from mold invasion nor reduce mold growth within the seed. This was proven more conclusively in the following test.

Influence of Various Solid Fungicides on the Development of Molds within Moist Seeds. Duplicate portions of nondelinted cottonseed, variety Bobshaw, obtained from Virginia, were conditioned to 19% moisture, dusted with five different fungicides, each in a concentration of one part fungicide per 100 parts of moist seed, and stored in rubber-stoppered 250 ml. Erlenmeyer flasks at room temperature for 30 to 60 days. In all cases, the amount of fungicide was in excess of that

which would adhere to the seed after vigorous shaking. The fungicides used were Arasan, Chloramine-B, Spergon, thiourea, and 8-hydroxyquinoline. Of these, thiourea, 8-hydroxyquinoline, and Chloramine-B were among the most effective mold inhibitors of more than 100 compounds tested by Milner, Christensen, and Geddes (17) on wheat seed. Arasan and Spergon are widely used and effective seed-treatment fungicides. They were included not because they were considered to be effective mold inhibitors on stored seed, but only to determine the influence of such compounds of known fungicidal potency upon storage molds.

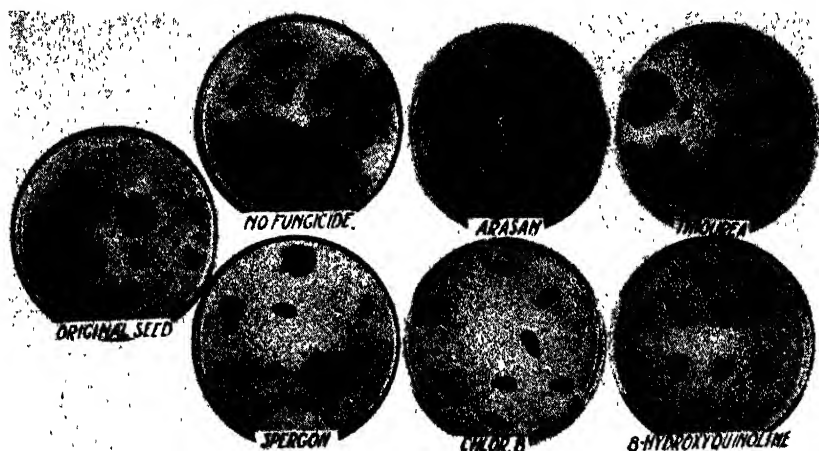


Fig. 4. Cottonseed treated with various dust fungicides (1:100) and stored 28 days at room temperature, 19% moisture, then delinted, surface disinfected, and cultured. Note that the kinds of molds infecting seed treated with thiourea differ from those from the seed treated with Arasan.

The total mold population after 30 and 60 days' storage and the percentage of seed with internal molds after 30 days' storage are shown in Table IV. None of these compounds entirely prevented mold increase, and some apparently stimulated mold growth. Some molds, especially *Aspergillus glaucus*, developed so vigorously on the seed treated with Arasan that the sporophores grew out in long tufts with visible particles of the fungicide on them. The mold population given in Table IV includes only the molds that developed internally. The seed was delinted in concentrated sulfuric acid before grinding, and this eliminated the heavy crop of external spores on some of the seeds. This treatment was necessary because, while a given compound might not inhibit molds on the seed at 19% moisture content, it might suppress their growth in agar; i.e., the potency of a given compound as determined by tests on agar plates may bear little relationship to its

potency under other conditions, a fact so well recognized as to need no emphasis. The internal mold infection in some of the seeds in this test is shown in Fig. 4.

Approximately 20 seeds treated with each compound were sectioned after 60 days' storage and examined with a binocular microscope for internal evidence of molds. Some internal mold was found in all of the

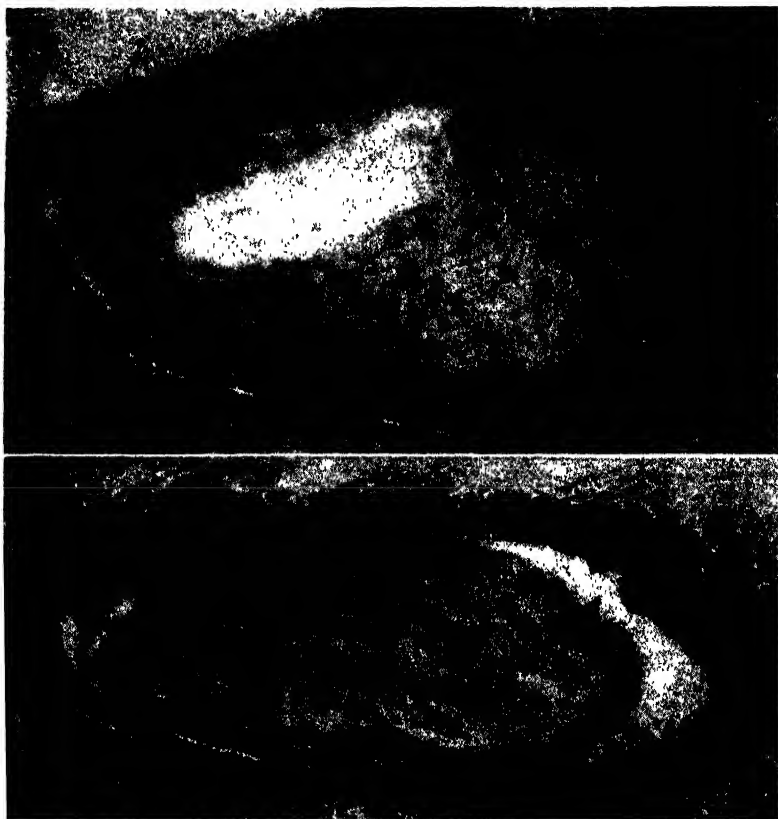


Fig. 5. Sections through seed treated with fungicide (top, Arasan; bottom, thiourea) and stored 60 days at 19% moisture. The arrows point to heavy mold development on the inner wall of the seed coat and on the embryo. The dark circular spots in the embryo are oil glands.

seeds and, in some, treated with supposedly effective moldicides, the entire inner wall of the seed coat was covered with mold sporophores. Photomicrographs of sections of these seeds are shown in Fig. 5.

That the seeds treated with 8-hydroxyquinoline, when cultured, appeared to be free of internal molds may have been due to small amounts of the compound that remained in the seed coat, sufficient to prevent the molds internally present from growing out in agar. As

evidence of this, such seed, when ground and cultured, gave rise to numerous mold colonies. All of these colonies arose from small portions of the seed coat, as determined by microscopic examination of the culture plates. These colonies appeared only after 10 to 14 days, grew very slowly, and were obviously suppressed. This suggests that the seeds treated with this compound were, at the end of the test, somewhat more moldy than the results indicated, and also that it may sometimes be rather difficult to determine whether molds are growing in seed treated with a fungicide.

It is evident from this test that compounds of even rather high toxicity to molds may be relatively ineffective in protecting moist cottonseed from invasion by storage molds. This is important because in some tests with stored cottonseed it has been assumed that treatment with a compound known to be toxic to certain fungi under certain conditions was also equally toxic to the storage molds, and that application of such compounds to the seed automatically eliminated molds. The present tests indicate that the effectiveness of a compound in eliminating molds from moist stored cottonseed can be determined only by testing the compound under the storage conditions involved, and by determining the mold population at the beginning and end of the test. Obviously, molds cannot be excluded as a factor in such studies and some sort of mold assay must be an integral part of experiments that aim to determine whether molds are involved in the deterioration of stored seeds.

Structure of Cottonseed in Relation to Mold Invasion. That the heavy, thick coat of cottonseed does not protect the interior of the seed from invasion by molds has already been suggested. The fact that some fungus diseases of the cotton plant are seed-borne, and the causal organisms have been found within the seed (3), would lead one to suspect that this seed is not so impervious to mold invasion as it seems.

There are numerous studies of embryo development and seed structure of the cotton plant, but none of them, so far as the authors have been able to determine, discuss the morphology of the seed from the standpoint of its possible invasion by fungi. In a study on this aspect of the problem mature seeds of several varieties of cotton were examined, and some plants of the Stoneville variety were grown from seed to maturity in the greenhouse to obtain seed in various stages of development.

The embryo of cottonseed develops anatropously, as has been shown by Gore (9) and others; that is, the seed is curved back against, and fuses with, the stalk on which it is borne, and through which its nourishment is obtained. As determined by dissection of numerous seeds at various stages of growth, the vein through which the seed is

nourished passes up one side of the seed and divides near the blunt end. Beneath this division of the vein there is a pore-like opening in the heavy seed coat through which a branch of the vein apparently passes. A diagrammatic sketch of a section through the basal portion of an almost mature seed is shown in Fig. 6. This is typical of all the seeds of the several varieties that have been examined. The significant fact from the standpoint of fungus invasion is that this pore in the mature seed is filled only with thin-walled parenchyma cells and presents to molds an easy access to the interior of the seed. The thin-walled parenchyma cells form a plug in the opening through the seed coat. In the immature seed, the cells of this plug are contiguous to, and continuous with, the cells of the thick endosperm that surround the embryo. As the seed matures and the endosperm is consumed, a cap of this

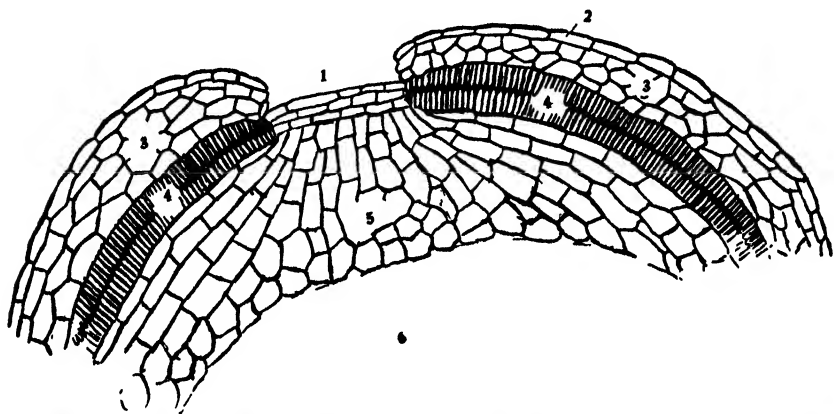


Fig. 6. Diagrammatic sketch of a section through the basal portion of a nearly mature cottonseed. Magnification about $\times 20$. (1) represents the hilum plugged with thin-walled cells, (2) epidermis, (3) mesophyll, (4) palisade cells, (5) endosperm (which remains as a cap over the embryo), and (6) embryo.

parenchyma tissue is left at the base of the embryo. When the ripe seed dries out, the cells of this cap separate from the inner wall of the seed coat, and frequently the plug of tissue retracts from the pore in the wall. In seeds that have been delinted with sulfuric acid, the open pore in the wall often is visible to the naked eye. Somewhat magnified, it appears as shown in Fig. 7. Sometimes this pore is concealed by a few very thin layers of cells that originally made up the outer epidermis of the seed. In none of the hundreds of individual mature seeds of several different varieties of cotton that have been dissected was there anything more than a superficial covering over this pore. Thus the interior of the seed is exposed to easy infection by such molds as may have opportunity to invade it.

As evidence that molds normally enter the seed through this readily available opening, spores of *Glomerella gossypii* have been

rather commonly found in the embryo tissue beneath the pore of high-quality seeds furnished the authors by the Southern Regional Research Laboratory. In cottonseed stored at 90% relative humidity for as short a time as 10 days, molds were found sporulating vigorously *within* the seed coat adjacent to this pore. With longer storage, at

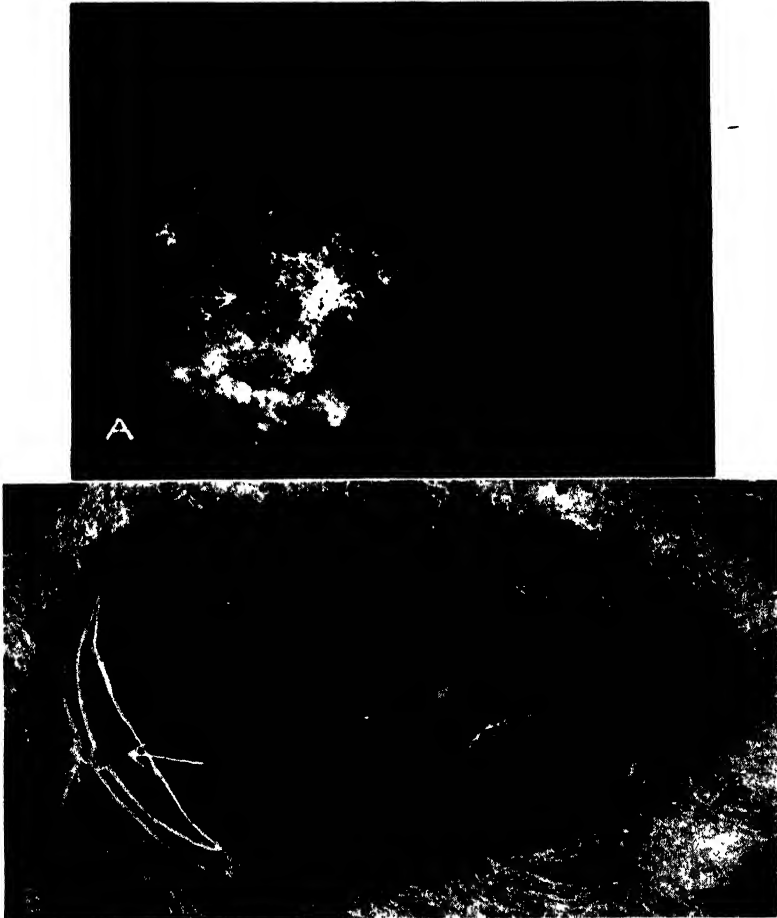


Fig. 7. A. Exterior of basal end of delinted cottonseed, showing the aperture (indicated by the arrow) in the seed coat. B. Section through a cottonseed showing the "cap" of tissue (outlined in white) which was pulled away from the seed coat, leaving an opening through the seed coat, indicated by the upper arrow.

moisture contents favorable for their development, the molds may spread over the entire inner wall of the seed coat and sporulate profusely within the seed coat.

The general impermeability of the seed coat may even promote mold development at times. Such a seed coat, of course, excludes

nonvolatile fungicides and doubtless limits the penetration of some liquid fungicides. Such fungicides may have little or no effect on molds growing within the seed coat. This helps to explain why compounds known to be toxic to molds, such as thiourea, Spergon, or Arasan, when applied to the outside of the seed, may not eliminate or even reduce mold growth within the seed. Thus, the chief difference between cottonseed and such seeds as wheat or soybeans, so far as mold invasion is concerned, is that heavily molded seeds of wheat or soybeans are likely to be outwardly and visibly moldy (although this is not always true), while cottonseed may be heavily molded internally even when it appears outwardly to be free of molds.

Respiration of Intact Seeds, Separated Meats, and Hulls. The hulls were removed by hand from approximately 400 g. of delinted cottonseed. That this resulted in relatively little injury to the embryos is shown by a germination of 85% of the separated meats, as compared with an initial germination of 95% of the intact seed. Meats and hulls were each divided into duplicate lots, and one of each was moistened with water, the other with a solution of thiourea to give one part thiourea per 100 parts of dry hull or meat. Duplicate portions of intact seed were similarly treated. The materials were stored at 30°C. for 10 days, and were continuously aerated with atmospheres of 90% relative humidity. The carbon dioxide production was measured daily, and the fat acidity, viability, and mold population of the seed after 10 days' storage. The results are given in Table V.

In this experiment, the solution of thiourea inhibited respiration and mold increase more effectively than did thiourea when applied as a dust (Table IV), but it did not eliminate molds. Respiration and mold population were considerably higher for the nontreated material than for that treated with thiourea. Molds increased slightly on the treated intact seed and on meats, but increased considerably on the treated hulls. That is, even applied as a solution, thiourea greatly reduced but did not completely inhibit mold increase. The major part of the respiration of the meats treated with thiourea must be attributed to the embryo itself, while the respiration of the hulls, both treated and untreated, must be ascribed mainly or solely to molds. The high respiration and high mold population of the nontreated meats indicate that the embryo, as well as the hull, is a favorable medium for mold increase. The test does not disclose whether the decrease in viability of the meats treated with thiourea was due to the toxicity of thiourea, to chemical processes within the embryo itself, or to molds that were invading the interior of the meats but which did not sporulate on the exterior and were not revealed in the mold assays. No attempt was made to determine the acidity of the hulls, since Karon (10) has

shown that they are essentially free of fats. Both the intact seeds and the separated meats show a close relationship between mold increase and fat acidity increase, indicating that molds may be significantly concerned in the deterioration of moist stored cottonseed.

TABLE V

CARBON DIOXIDE PRODUCTION, FAT ACIDITY, AND MOLD POPULATION OF INTACT MOIST COTTONSEED, COTTONSEED MEATS, AND HULLS, STORED AT 90% RELATIVE HUMIDITY WITH AND WITHOUT THIOUREA

(Samples stored 10 days at 30°C.)

Material	Treatment	Moisture content		Respiration ²	Fat acidity		Germination	Molds per g.	Principal kinds of molds
		Original	Final ¹		As oleic	Acid value ³			
Original seed	Nil	—	—	mg.	%	mg.	%		
Original meats	Nil	—	—	—	0.5	10.0	90	1,000	<i>Glomerella gossypii</i>
Intact seed	Nil	19.2	21.0	1116.6	4.4	92.5	64	5,500,000	<i>Aspergillus glaucus</i> (probably from contamination during sampling)
	Thiourea	17.9	18.1	246.3	0.6	13.8	95	5,000	<i>A. glaucus</i> and <i>Penicillium</i> sp.
Meats alone	Nil	17.9	19.9	5443.6	14.2	301.8	0	100,000,000	<i>A. glaucus</i> and <i>A. candidus</i>
	Thiourea	17.3	17.7	1057.4	0.3	5.6	50 ⁴	1,000	<i>Penicillium</i> sp.
Hulls alone	Nil	19.8	19.5	858.7	—	—	—	2,500,000	<i>Penicillium</i> sp. and <i>A. glaucus</i>
	Thiourea	19.5	18.9	192.4	—	—	—	140,000	<i>Penicillium</i> sp. and <i>A. versicolor</i>

¹ According to Karon (10), at 90% relative humidity the meats could be expected to have a moisture content of 18.0%, the hulls 20.0%, and the intact seeds 19.2%. The moisture content of the non-treated seeds, meats, and hulls increased during the test, probably chiefly as a result of mold growth.

² Total mg. CO₂/100 g. dry matter.

³ Expressed as mg. KOH/10 g. of oil.

⁴ No normal sprouts developed from these meats

Discussion

The results clearly show that an increase in mold population in moist stored cottonseed is accompanied by increases in the production of carbon dioxide, fatty acids, and heat, as has been found to be the case by workers in several laboratories with other kinds of seeds. In dry grain, the respiratory rate is very low. As its moisture content is raised, the respiration increases very gradually until a certain critical

moisture range is reached, above which marked acceleration in the rate occurs and heating tendencies appear along with an increase in fat acidity. This sharp break in the moisture-respiration curves and concomitant increases in heat production and fat acidity, observed when continuous optimum aeration is employed, occurs at moisture values for the various grains which are in equilibrium with a relative humidity of about 75%, the lowest value at which the most xerophytic of the molds commonly found on, and in, grain will grow readily. Bacteria are not normally a factor in the spoilage of stored grain since the moisture content must approach equilibrium with an atmosphere exceeding 95% relative humidity before they will grow readily, and the authors attribute the sharp increase in respiration, heat production, and fat acidity which occurs above the critical moisture value to mold growth. That is, at moistures below the critical value, the low and rather uniform rate of carbon dioxide production is due chiefly to the physiological activity of the viable seeds, but at higher moistures, the seed respiration is supplemented by the much greater respiration of the growing molds. That the increase in fat acidity at high storage moistures is due mainly to the action of mold lipases rather than to the activation of the lipases of the cottonseed itself, as postulated by research workers at the Southern Regional Research Laboratory (1, 11, 12, 14), is indicated by the effect of differential aeration at constant moisture. Under nitrogen practically no mold growth occurred, and there was a negligible increase in fat acidity over the period of the trials. As the aeration was increased, there was a concomitant increase in the mold population and in fat acidity. That molds may produce lipase in quantity has been shown by Eyre (7), Kirsh (13), Thibodeau and Macy (24), Nagel and Semeniuk (21), and others. The researches of Nagel and Semeniuk are of particular interest in relation to the origin of the increase in fat acidity upon storing high-moisture grain. These workers grew pure cultures of nine fungi (which had been isolated from naturally molded corn) on steam-sterilized corn containing about 32% moisture and found that all the fungi increased the fat acidity of the corn. The greatest increases were produced by *Penicillium chrysogenum* I, *P. chrysogenum* II, *Aspergillus niger*, *A. flavus*, *Mucor racemosus*, and *A. amstelodami*. Unpublished work by J. J. Goodman at the Minnesota Experiment Station has also shown that many of the storage molds encountered on agricultural seeds produce lipase.

In several of the present studies, molds increased from an original population of less than 1,000 per gram to a final population of millions per gram during a storage period of only 10 to 20 days. Since several of these molds are known to produce lipase, and since cottonseed itself

has not been proven to produce lipase under the storage conditions maintained in these tests, it seems reasonable to attribute a considerable proportion of the fat acidity increase in these stored seeds to the action of mold lipase. The parts played by seed enzymes and mold enzymes in the deteriorative process admittedly can be determined only by tests in which the seeds are stored free of molds. Such tests are difficult to make, primarily because it is difficult to keep seed free of storage molds under conditions that favor their growth. To eliminate molds it is not sufficient merely to treat seed with a supposed fungicide. Regardless of the means used to eliminate or inhibit molds, one must depend upon some method of measuring mold population to determine experimentally whether molds are increasing or decreasing. The techniques used for this purpose in the experiments described here are by no means perfect, but at least they permit a fair quantitative comparison of moldiness. Neither Malowan (15), who stated that molds were not involved in the deterioration of cottonseed, in his tests, nor Altschul and his associates (1, 5), who expressed doubt regarding their role in the deterioration of this seed on storage, presented any experimental evidence to indicate whether the mold populations in the seeds they studied were increasing or decreasing. The data presented here leave little room for doubt that molds were a significant factor in the production of carbon dioxide, heat, and fatty acids in these experiments, and imply that they may have had an equally important, if unobserved, part in somewhat similar tests made by other workers.

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DETERMINATION OF THE PENTOSANS OF WHEAT AND FLOUR AND THEIR RELATION TO MINERAL MATTER ¹

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ABSTRACT

A study was made of the correlation between pentosan and ash content and the possible use of pentosan values as a criterion of flour grade. Consideration was given to methods of pentosan analysis.

The Hughes-Acree method for determining pentosans was adaptable to wheat and flour. Significant correlations between ash and pentosan content of two series of flours experimentally milled to varying degrees of purity were $r = +0.85$ and $r = +0.56$. However, clear grade flours showed lower correlations. The analyses of flour mill streams showed that low pentosan was generally associated with low ash content. Pentosan content was found generally to decrease with flour particle size. Among the individual bran layers the pentosan content was highest in the epidermis portion, and a significant correlation between ash and pentosan of $r = +0.63$ was found for epidermal tissues obtained from various wheats. No significant correlation was found for the cross layer, testa, or hyaline-aleurone layers, since the ash of these layers varied greatly among the varieties of wheat studied, whereas the pentosan content was fairly uniform.

The purposes of this investigation were to find a method suitable for routine determinations of pentosans of wheat and flour and to investigate the possibility that pentosan content is correlated with the mineral matter or ash content. Additional information concerning the distribution of pentosans in wheat and flour may lead to the practical use of the pentosan determination as a flour-grading factor.

Jacobs and Rask (5) suggested the use of pentosan content in calculating flour yield and reported pentosan values from 2.95 to 3.20% on a 13% moisture basis. Baker, Parker, and Mize (1) claim that two types of pentosans exist in wheat flour: a water-soluble type that forms a gel when oxidized and thus may have a role in baking, and an insoluble type that is present in relation to the surface area of the small starch granules.

Generally, existing methods of determination depend upon the hydrolysis of pentosans with a solution of hydrochloric acid and distillation of the furfural produced from the pentose sugars. Empirical factors are applied to the furfural determination and the results expressed as pentosans. The accuracy is limited by the yield of furfural, loss by volatilization, and destruction by overheating. For example,

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production of furfural derivatives, particularly hydroxymethylfurfural from glucose, will cause an error of overestimation. Often errors cancel one another, but results from abridged determinations require comparison with official methods to assure accuracy.

Gravimetric methods such as the phloroglucinol and thiobarbituric acid precipitations which require overnight to complete them are not adaptable to a large number of analyses. Volumetric methods have the advantages of speed and sensitivity at low concentration of pentosans. Noll and Belz (8) describe a method involving the use of hydroxylaminehydrochloride. The reaction involves the formation of α -furanaldoxime and hydrochloric acid which can be titrated with standard alkali. The work of Hughes and Acree (2, 3, 4) on the use of controlled bromination of furfural is particularly noteworthy as the method is easily adapted to quick routine analysis. Vernon and Metzner (10) modified the procedure and reported it adaptable to rye, corn, barley malt, and distillers' dried grain.

Materials and Methods

The apparatus used for hydrolysis of pentosans and distillation of furfural consisted of a 500-ml. flask fitted with a two-hole stopper. A bent tube directed toward the inside wall of the flask was connected to a separatory funnel graduated in 30-ml. intervals so that the acid concentration could be kept constant. A connecting bulb linked the flask to a condenser. The distillate was passed through a small filter and collected in a 500-ml. glass-stoppered Erlenmeyer flask marked in 30-ml. graduations.

A weighed sample was introduced into the boiling flask with some glass beads, and 125 ml. of 12% hydrochloric acid solution (by weight). Heat sources were 300 watt Goldfish units connected in pairs in parallel circuit, giving distillation rates of 30 ml. per 10 minutes with a minimum of local charring. Distillation was continued until 360 ml. of distillate were collected, 30-ml. portions of the hydrochloric acid solution being added to the boiling flask as equivalent amounts of distillate were collected.

Studies were made of distillates from samples of bran and commercial patent flour using the phloroglucinol method as described in *Cereal Laboratory Methods* (4th ed., 1941), the hydroxylaminehydrochloride method, and a modified bromine method. The modified bromine method is as follows:

The flasks containing the distillate were closed and cooled in an ice bath to 0°C., 25 ml. of bromide-bromate solution (3 g. of potassium bromate and 50 g. of potassium bromide per liter) were introduced by a fast-delivery pipette and the flask closed quickly. The reaction was

allowed to continue for exactly 4 minutes, 10 ml. of 10% potassium iodide solution added, the flask closed and shaken gently.

The solution was titrated rapidly with 0.1 *N* sodium thiosulfate solution to a yellow color, starch indicator added, and titration continued to a colorless end point. A blank determination was made in the same manner. The pentosan content of the sample in grams equals ml. of 0.1 *N* sodium thiosulfate (blank minus titration) \times 0.0082. All determinations are averages of duplicates.

The method of Shetlar, Rankin, Lyman, and France (9) was used with slight modification to remove the individual bran layers from 10 varieties of hard red winter wheat for a study of the distribution of pentosan and mineral matter. The epidermis and cross layer fractions were virtually pure, but the testa contained some contamination from these layers. The microscopic technique of Shetlar *et al.* was applied to the testa fraction for correction of the percentage of the kernel represented by epidermis, cross layer, and testa, but was not applied to the results of chemical tests.

The hyaline-aleurone fraction contained some endosperm, and all analyses were corrected to an endosperm-free basis by starch analyses since pure hyaline-aleurone is free of starch. Starch was determined by the polarimetric method described in *Cereal Laboratory Methods* (5th ed., 1947).

The epidermis, hyaline-aleurone, and endosperm fractions were ashed as recommended in *Cereal Laboratory Methods* (5th ed., 1947), but the cross layer and testa portions required special treatment. In the latter case, ash was obtained at 600°C. after pretreatment with glycerol-alcohol mixture as described in *Cereal Laboratory Methods* (4th ed., 1941). The sodium content of the ash from cross layer, testa, and endosperm samples was determined by the spectrographic method of Morris, Pascoe, and Alexander (7) and the results, calculated as sodium carbonate, were deducted from the corresponding ash values

TABLE I
COMPARISON OF THREE METHODS OF PENTOSAN DETERMINATION
ON WHEAT BRAN AND FLOUR

Sample	Pentosan content		
	Phloroglucinol	Hydroxylamine-HCl	Bromine
Bran	%	%	%
	21.7	22.6	21.6
	21.1	21.7	21.2
Flour	2.90	3.14	2.96
	2.92	3.20	2.96

to correct for the treatment with alcoholic sodium hydroxide. Because of the great bulk of the epidermis and the difficulties in determining moisture, samples of this fraction were air-dried in an air-conditioned room for 48 hours to attain moisture equilibrium. The samples were stored in sealed cans until analyzed.

Samples of the original and debranned wheat varieties were milled on the Buhler mill after tempering to 15.5% moisture and the flours analyzed for ash and pentosan. The debranned wheat had the epidermis, cross layer, and testa layers removed, leaving only the hyaline-aleurone layers attached to the starchy endosperm.

Results and Discussion

The pentosan results obtained by the hydroxylaminehydrochloride, modified bromine, and phloroglucinol methods on bran of Pawnee variety wheat and commercial hard wheat flour are shown in Table I. The hydroxylamine method gave the highest results but the poorest precision. The necessity of exactly neutralizing the acid-furfural distillate with methyl orange made this method impractical for a large number of analyses.

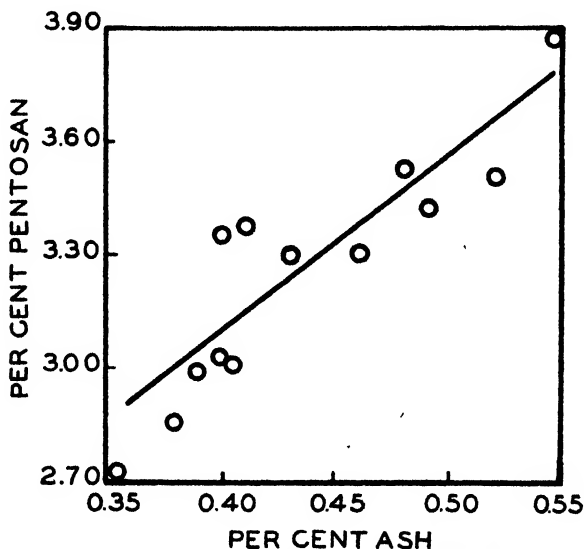


Fig. 1. Scatter diagram showing relation between pentosan and ash content of flours experimentally milled from hard red winter wheat to varying degrees of purity.

The bromine method gave comparable results to the phloroglucinol method. The difference between duplicates for the bromine method was less than 2% and the total time of determination was less than 3 hours. To obtain the maximum yield of furfural at least

360 ml. of distillate had to be collected, since marked underestimations were noted with lesser amounts. Nevertheless the bromine method was adopted for the following studies.

Pentosan determinations were made on 13 flours which were experimentally milled to varying degrees of purity from hard red winter wheat, to give samples covering a similar range in ash content to that encountered in commercial flours. Fig. 1 shows the relationship between ash and pentosan content of these flours. The significant correlation coefficient of $r = +0.85$ suggested that the pentosan value

TABLE II
ASH AND PENTOSAN CONTENTS OF FLOUR STREAMS MILLED
FROM PAWNEE VARIETY HARD RED WINTER WHEAT¹

Sample	Ash	Pentosan
	%	%
MILLED FEBRUARY 25, 1947		
Third break	0.44	2.23
Sizing	0.32	2.33
First middling	0.33	2.51
Second middling	0.28	2.41
Third middling	0.32	2.57
Fourth middling	0.36	3.00
Flour	0.40	3.00
MILLED MARCH 3, 1947		
First break	0.54	2.17
Second break	0.55	2.65
Third break	0.51	2.62
Fourth break	0.72	2.58
Sizings	0.33	2.57
First middling	0.31	2.45
Second middling	0.27	2.24
Third middling	0.30	2.38
Fourth middling	0.35	2.83
Fifth middling	0.53	3.29
First tailing	0.54	2.51
Second tailing	0.55	2.94
First and second reels	0.65	3.15

¹ Results reported on a 14% moisture basis.

might be used to predict ash content. However, 12 clear flour samples with ash contents ranging from 0.60 to 1.16% gave the nonsignificant correlation of $r = +0.14$. In flour samples having ash contents greater than approximately 0.55%, the pentosan values were found to fluctuate greatly.

Ash and pentosan analyses made on samples of certain mill streams collected on two days from the 130-sack mill at Kansas State College while operating experimentally on Pawnee variety wheat are recorded in Table II. Analogous conditions existed during both samplings.

The first break stock, which is high in ash, and the first, second, and third middling streams, which are low in ash, are among the lowest in pentosan content. Thus contradicting results appear. However, the high-ash streams such as the fourth and fifth middlings, low grade, and second tailings stock were high in pentosans. Statistical analysis of the results gave a nonsignificant correlation ($r = +0.39$) that could not be used for predicting ash content from pentosan determinations.

Ash and pentosan analyses of the flour produced from 10 varieties of hard red winter wheat showed a slightly significant correlation of $r = +0.56$ between the two determinations. However, flour from the same wheats milled after being debranned gave no significant cor-

TABLE III
ASH AND PENTOSAN CONTENT OF HYALINE-ALEURONE LAYER
AND ENDOSPERM FOR SEVERAL WHEAT VARIETIES¹

Variety	Ash			Pentosan		
	Hyaline-aleurone	Flour from debranned wheat	Flour from original wheat	Hyaline-aleurone	Flour from debranned wheat	Flour from original wheat
	%	%	%	%	%	%
Tenmarq	7.26	0.56	0.59	23.5	2.45	2.84
Wichita	8.38	0.59	0.54	23.3	2.31	2.84
Comanche	8.11	0.65	0.55	21.0	2.21	2.74
Pawnee	5.70	0.62	0.56	22.9	2.04	2.49
E. Blackhull	6.05	0.52	0.53	23.9	2.51	2.88
Kawvale	4.78	0.55	0.49	23.6	2.37	2.86
Westar	4.73	0.52	0.44	25.0	2.40	2.62
Triumph	6.01	0.51	0.45	26.1	2.14	2.69
Red Chief	6.59	0.55	0.49	25.3	2.37	2.94
Chiefkan	6.72	0.53	0.53	23.8	2.49	3.02

¹ Results reported on a 14% moisture basis.

relation between ash and pentosan content. The pentosan values were consistently lower than previously encountered in flours of similar ash range.

The milling characteristics of the debranned wheat differed from the original wheat, and the absence of the outer bran layers from the wheat has reduced the pentosan content of the flour as shown in Table III. Baker *et al.* (1) reported approximately 0.06 g. of soluble pentosans per 5 g. of flour. This is about twice the difference between the pentosan values of the flour from the original and debranned wheat. The reduction in pentosan content of the endosperm from debranned wheat probably results from the combination of conditions including less contamination by bran layers, since the epidermis, cross

layer, and testa are removed, and the loss of some soluble pentosans during the process of debranning.

Relation of Particle Size to Pentosan Content. A hard red winter wheat flour of 0.47% ash content was divided into eight particle size groups by the method of Wichser *et al.* (11) and the eight fractions were analyzed for pentosans. As shown in Fig. 2, no consistent relationship between particle size and pentosan content was found. The 105 to 88 μ fraction contained the most pentosan and, in general, lower values were found as the particle size decreased except for the 61 to 53 μ range. Microscopic examination of this fraction did not show excessive amounts of bran contamination.

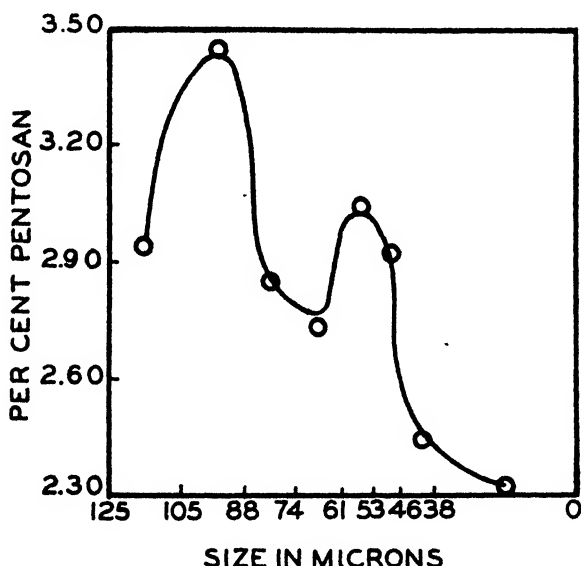


Fig. 2. Relationship between particle size and pentosan content for hard red winter wheat flour.

Baker, Parker, and Mize (1) reported 33 times as much water-insoluble pentosan in the small starch particles as in the large starch granules. MacMasters and Hilbert (6) also reported about 4% pentosans in the small granule fraction of Turkey wheat flour. In the present investigation an effort was made to study the small granular fraction of flour by employing a sifting method, but no attempt was made to separate starch granules. It was assumed that when a substance of heterogeneous particle size is sifted, the smaller particles pass through the sieve openings first. On the basis of this assumption and the results of previous investigators (1, 6) the first material through a 0-38 μ sieve should show a higher pentosan value than subsequent sievings.

To test this contention the flour used in this study was sifted 30 seconds on a Ro-Tap sifter and the throughs collected. The process was continued for another 30 seconds and thereafter repeated at one-minute intervals for a total of 5 minutes. Fig. 3 shows that the greatest amount of pentosan was found at the end of the second minute, followed by a decrease as sifting time increased. It is apparent from Figs. 2 and 3 that the smallest flour particles do not have the highest pentosan content. A satisfactory relationship between particle size and pentosan content was not established by these studies.

Individual Bran Layers and Endosperm. Pentosan and ash determinations were made on the different bran layers and endosperm

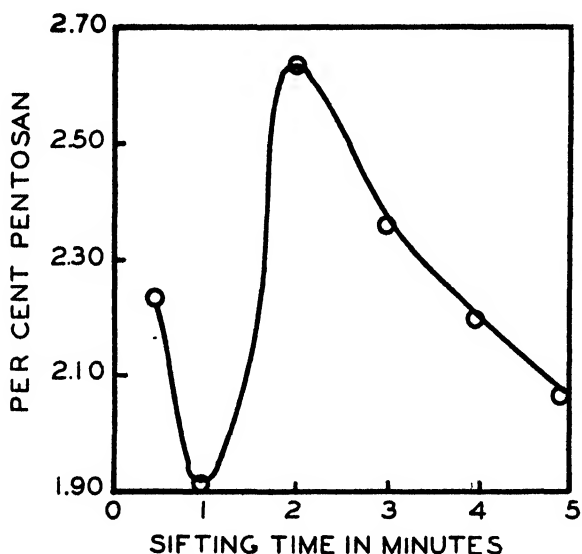


Fig. 3. Effect of sifting time on the pentosan content of flour sifted through a 400-mesh wire sieve. Particles up to $38\ \mu$ in diameter can pass this sieve.

which were separated by the procedure of Shetlar *et al.* (9). The amount of each layer, expressed both as percentages of the kernel and of total bran, is shown in Table IV. The values for each variety are the means of 10 separations and are in agreement with those of Shetlar and associates.

In separating the tissues, varietal differences were observed. For example, the epidermis of Early Blackhull required more severe treatment to remove it than was the case for the other varieties. The cross layer of Early Blackhull was easily removed, but the separation of the testa layer required extra stirring. Similar observations were made during removal of cross layer and testa from Red Chief and Chiefkan.

TABLE IV
PERCENTAGES OF INDIVIDUAL BRAN LAYERS IN 10 VARIETIES
OF HARD RED WINTER WHEAT¹

Variety	Epidermis		Cross layer		Testa		Hyaline-aleurone		Bran
	Kernel	Bran	Kernel	Bran	Kernel	Bran	Kernel	Bran	Kernel
	%	%	%	%	%	%	%	%	%
Tenmarq	3.4	23.1	0.6	3.9	0.4	2.6	10.4	70.4	14.8
Wichita	3.1	22.1	0.7	4.6	0.2	1.4	10.2	71.9	14.2
Comanche	3.5	—	0.7	—	0.3	—	—	—	—
Pawnee	3.4	23.7	0.8	5.7	0.4	3.1	9.7	67.5	14.3
Early Blackhull	3.3	22.8	1.5	10.1	0.4	3.0	9.6	64.6	14.9
Kawvale	2.7	20.8	0.8	6.3	0.5	3.7	9.1	72.4	13.2
Westar	2.6	20.0	0.6	4.8	0.3	2.5	9.6	72.7	13.1
Triumph	2.9	20.3	0.9	6.0	0.6	4.2	10.1	69.5	14.5
Red Chief	3.1	23.4	0.9	7.0	0.6	4.7	8.6	64.9	13.2
Chiefkan	3.3	—	0.5	—	0.2	—	—	—	—
Average	3.1	22.0	0.8	6.1	0.4	3.1	9.7	69.2	14.0

¹ Percentages are expressed both on basis of the total bran and of the entire kernel.

These characteristics were attributed to the waxy nature of the bran layers of these varieties. In contrast, Kawvale and Westar, because of the ease with which the layers separated, required careful handling to avoid contaminating the individual layers.

The pentosan and ash contents of 10 varieties of wheat and of their epidermal tissues are recorded in Table V. The correlation between wheat ash and pentosan content was nonsignificant ($r = +0.25$), and for the air-dried epidermis fractions a significant coefficient of $r =$

TABLE V
COMPARISON OF ASH AND PENTOSAN CONTENT OF SEVERAL VARIETIES OF
WHEAT AND THE AIR-DRIED EPIDERMIS OF THE SAME WHEATS¹

Variety	Wheat		Air-dried epidermis	
	Ash	Pentosans	Ash	Pentosans
	%	%	%	%
Tenmarq	1.75	7.4	0.90	44.0
Wichita	1.73	7.2	0.94	43.6
Comanche	1.71	7.8	0.90	43.6
Pawnee	1.60	7.1	1.00	42.7
Early Blackhull	1.83	7.4	1.20	48.4
Kawvale	1.78	7.1	1.49	46.7
Westar	1.62	6.9	1.08	46.6
Triumph	1.66	7.3	0.98	47.3
Red Chief	1.61	7.8	0.96	46.8
Chiefkan	1.70	7.6	0.93	45.0

¹ Results reported on 14% moisture basis.

+0.63 was obtained. The ash content of the epidermis varied from 0.90% to 1.49% and the pentosan values from 42.7% to 48.4%; the latter are higher than previously reported, but repeated sampling confirmed these results.

The data for the epidermal layer indicate a possible reason for the low correlation between ash and pentosan in clear flours. The range in ash content of the epidermis was only slightly higher than that of the clear flours, but the pentosan values for the epidermis are approximately 10 times as great as those for clear flour. The contamination of these flours with small amounts of bran in the form of "beeswing" or epidermis would influence the pentosan value far more than the ash content.

TABLE VI

PENTOSAN AND ASH CONTENTS OF COMPOSITE SAMPLES OF CROSS LAYER AND OF TESTA TISSUES FOR 10 WHEAT VARIETIES¹

Variety	Ash		Pentosan	
	Cross layer	Testa	Cross layer	Testa
	%	%	%	%
Tenmarq	13.71	13.18	27.2	31.6
Wichita	11.15	13.32	30.2	29.4
Comanche	9.42	13.87	32.2	27.4
Pawnee	8.58	11.25	34.5	31.2
Early Blackhull	7.01	9.05	37.9	34.8
Kawvale	6.41	8.06	35.8	32.1
Westar	5.32	8.03	37.4	36.0
Triumph	6.38	7.40	41.5	35.8
Red Chief	6.42	6.96	36.6	34.0
Chiefkan	5.45	7.23	36.9	34.6

¹ Results reported on 14% moisture basis and the ash corrected for sodium hydroxide absorption.

The analyses of composite samples of cross layer and of testa tissues for ash and pentosans are given in Table VI. The pentosan data were consistent and in accord with previous reports. However, the ash values varied widely among varieties; Tenmarq, Wichita, Comanche, and Pawnee had notably higher ash values for these two layers. No significant correlation was found between ash and pentosan in cross layer and testa.

Table III shows the ash and pentosan results on hyaline-aleurone layers and endosperm from debranned and normally milled wheat. Sodium corrections were not necessary for the hyaline-aleurone ash values because of the acid-washing and neutralization steps, but corrections were applied to the values for flour ash. Ash and pentosan values of the hyaline-aleurone material reported in Table III were corrected for endosperm contamination. This can be done on the

basis of starch determination since pure hyaline-aleurone is void of starch. The corrected results agree with those previously reported and the values are relatively constant. The hyaline-aleurone layers constitute the major portion of the total bran and thus contribute the largest amount of ash and pentosan in the wheat kernel.

Acknowledgments

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EFFECT OF VARYING CONCENTRATIONS OF CERTAIN METALS AND THEIR SALTS ON GAS PRODUCTION AND LOAF VOLUME¹

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WILLIAM G. SCHRENK³

ABSTRACT

Spectrographic examination of filings from the planetaries of a new 100 g. bread dough mixer indicated the presence of cadmium. Cadmium was also detected spectrographically in the ash of bread baked from dough mixed in the new mixer. These cadmium-plated planetaries materially inhibited yeast fermentation, thereby resulting in reduced gas production and loaf volume.

Gas production and bread-baking studies were carried out with varying concentrations of cadmium and its chloride along with the salts of other important metals (chromium, nickel, tin, zinc, copper, magnesium, and manganese) used in plating and in aluminum and steel alloys. Salts of lead and mercury were included for academic reasons.

The enzymes of yeast are inactivated either completely or in part by certain of these metallic salts regardless of concentrations, and by others only at specific concentrations. As little as 1 p.p.m. cadmium chloride produces a material reduction in yeast activity and loaf volume.

Chromium, nickel, or tin, and preferably the first two, are biologically safe when used for plating surfaces that come in contact with yeast-fermented doughs.

Bread from dough mixed in a new mixer recently purchased for the Hard Winter Wheat Quality Laboratory was found to be decidedly inferior with respect to loaf volume, crumb characteristics, and flavor as compared with that from an older but similarly constructed mixer. When all pin and planetary surfaces of the new mixer were covered with scotch tape, however, normal baking results were obtained. Uncovering either the bowl pins or planetary pins resulted in no damaging effects to doughs. With the planetaries bare, however, and all pins covered, subnormal loaf volumes and underdeveloped crumb grains again were obtained. Cadmium was found spectrographically in the filings from the planetaries of the new mixer but not from the old mixer. In addition, cadmium was identified (after concentration by precipitation with hydrogen sulfide) in the bread baked from doughs mixed in

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² Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Departments of Chemistry and Milling Industry, Kansas Agricultural Experiment Station, Manhattan, Kansas. Contribution Nos. 369 and 155 from the Departments of Chemistry and Milling Industry, respectively.

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the new mixer but was not found in the bread when the old mixer was used. Simultaneously with the securing of these data, it was learned from the manufacturer that the planetaries of the new mixer had been plated with cadmium. Accordingly, bread-baking and gas production studies were carried out with varying concentrations of cadmium and its chloride along with the salts of other important metals used in plating and in aluminum and steel alloys. This paper presents the data obtained in these studies including certain comparisons of the old and new mixers.

Materials and Methods

A hard red winter flour composite of a number of typical varieties from each of two crop years was used for all baking and gas production studies. This flour, which was extracted on the Kansas State College 130 hundredweight mill, contained 11.3% protein and 0.47% ash.

Each bread dough contained 100 g. flour, 66.7 g. water, 6 g. sugar, 1.5 g. salt, 3 g. shortening, 2 g. yeast, 4 g. nonfat milk solids, 0.25 g. 120°L. malt syrup, and 1 mg. potassium bromate. All mixing except as otherwise specified was done in the old mixer—a National-Swanson-Working model. Mixing time was $2\frac{7}{8}$ minutes at 86 r.p.m. for the old mixer and $2\frac{1}{2}$ minutes at 100 r.p.m. for the new mixer. Fermentation periods and temperatures recommended by the A.A.C.C. were used. Concentrations of 0.001 mg., 0.01 mg., 0.1 mg., 1 mg., 10 mg., and 100 mg. of each of the metallic salts including cadmium chloride, chromic chloride, cupric chloride, mercuric chloride, magnesium sulfate, manganous chloride, nickel chloride, stannic chloride, zinc chloride, and lead acetate were added to the bread doughs.

Cadmium, chromium, nickel, tin, and zinc are used extensively for plating. These five metals together with copper, magnesium, and manganese are used in aluminum and steel alloys. The salts of lead and mercury were included in the experiments because of their academic interest.

The formula used in bread baking also was used in the gas production studies; 18.35 g. dough, or one-tenth of that used in bread baking, was scaled off from a regular size bread dough and placed in a pressuremeter with attached manometer. Thereafter the test was carried out as described in *Cereal Laboratory Methods*. Readings were taken every hour for 6 hours.

Results

Data in Table I show the relative toxicity of each of the various salts on loaf volume when present in the dough in concentrations of 0.001 mg. (1 part per 100,000,000) to 100 mg. (1 part per 1,000). Normal baking results were secured with concentrations of 10 mg. (1

TABLE I
LOAF VOLUMES OBTAINED WITH THE ADDITION OF VARYING AMOUNTS
OF METAL SALTS TO THE DOUGH

Concentration in dough	Metal salt added									
	CdCl ₂	CrCl ₃	CuCl ₂	HgCl ₂	MgSO ₄	MnCl ₂	NiCl ₂	SnCl ₄	ZnCl ₂	Pb(C ₂ H ₃ O ₂) ₂
mg.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
None ¹	807	807	807	807	807	807	807	807	807	807
0.001	796	—	800	804	815	805	810	825	805	799
0.01	788	818	795	799	805	800	795	830	810	808
0.1	742	819	785	806	825	810	810	810	810	808
1	580	797	798	801	800	820	796	810	761	806
10	548	805	778	674	810	808	790	794	520	789
100	543	794	460	235	805	819	745	741	761	746

¹ Loaf volume when mixed in new mixer was 674 cc. (equivalent to the effect of about 0.3 mg. or 3 p.p.m. of cadmium chloride).

part per 10,000) or less of the salts of chromium, copper (probably), magnesium, manganese, nickel, tin, and lead. Thus chromium, nickel, and tin should be satisfactory for plating surfaces that come in contact with yeast-fermented doughs. However, 10 mg. of each of the salts of cadmium, mercury, and zinc reduced loaf volumes 32%, 16%, and 36% respectively.

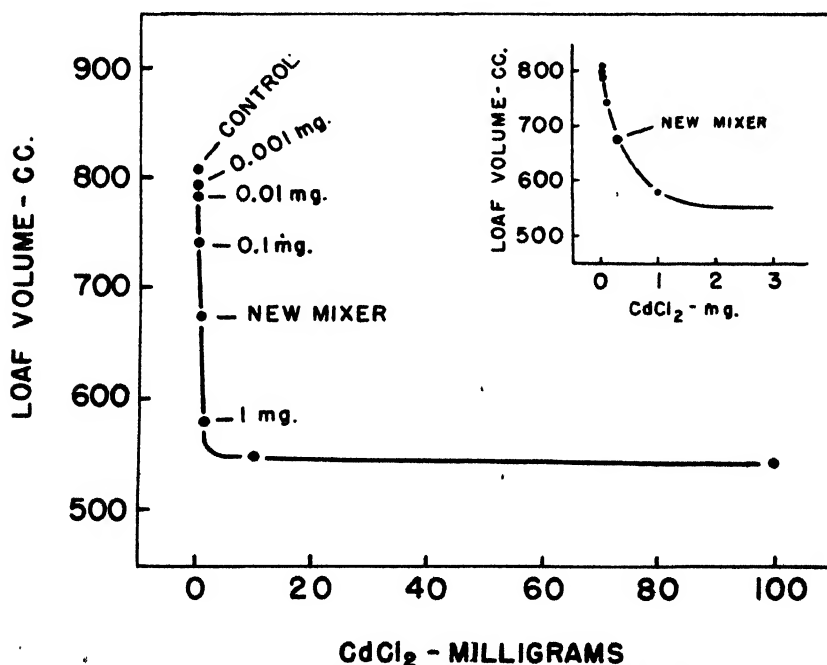


Fig. 1. Loaf volume of doughs containing varying concentrations of cadmium chloride and of dough mixed in new mixer (cadmium-plated planetaries).

At 100 mg. concentrations, mercuric chloride reduced loaf volume to 235 cc., a reduction of 71%. Enzyme activity appeared to be inhibited completely. Copper chloride, which had little or no effect on loaf volume at lower concentrations, reduced loaf volume to 460 cc., 43% below normal. Salts of nickel, tin, and lead, which at lower concentrations gave normal baking results, produced somewhat reduced loaf volumes at 100 mg. concentrations.

Several pertinent points concerning the relation of cadmium chloride concentrations in dough to loaf volume are illustrated in Fig. 1. For example, as little as 0.1 mg. of cadmium chloride (1 p.p.m.) produced a material reduction in loaf volume. When incorporated with high protein flours, even smaller concentrations of cadmium and its salts probably would result in material loaf volume reductions. Loaf volume continued to fall off rapidly, approaching the minimum as the concentration of cadmium chloride was increased to about 1.0 mg. Thereafter loaf volume decreased more slowly, the minimum volume of 550 cc. being attained with only 2 mg. cadmium chloride (Fig. 1 inset). Additional increases up to 100 mg. produced no further change in loaf volume. It should be noted from the inset of Fig. 1 that the loaf volume of 674 cc. for the new mixer corresponds to that expected for dough containing 0.3 mg. cadmium chloride or about 1.85 parts cadmium per million parts of flour.

The greatly reduced loaf volumes obtained from doughs containing cadmium, its chloride, or salts of copper, mercury, and zinc were accompanied by underdeveloped crumb grains and flavor. These results strongly indicated that dough fermentation had been seriously impaired. Accordingly, gas production studies were carried out on doughs containing 0 mg. (control), 1 mg., 10 mg., and 100 mg. of cadmium chloride and other important salts of metals used in plating and in steel and aluminum alloys.

Gas production data for the doughs containing 1 mg. of each of the various salts and for one dough mixed in the new mixer without any addition of salts are shown in Fig. 2. The only subnormal gas production curves were produced by mixing dough in the new mixer and by adding cadmium chloride to dough mixed in the old mixer. These data show that cadmium and its chloride salt are toxic to yeast fermentation. It is pertinent to note that of the five important plating materials studied (tin, nickel, chromium, zinc, and cadmium), only cadmium was toxic at 1 mg. concentration. The data are in good agreement with the loaf volume results.

Gas production data for doughs containing 10 mg. and 100 mg. of four salts, cupric chloride, mercuric chloride, zinc chloride, and cad-

mium chloride, together with those for the control and new mixer are plotted in Figs. 3 and 4, respectively.

Gassing data at the 10 mg. level (Fig. 3) are in excellent agreement with the loaf volume data in that they are about normal for cupric

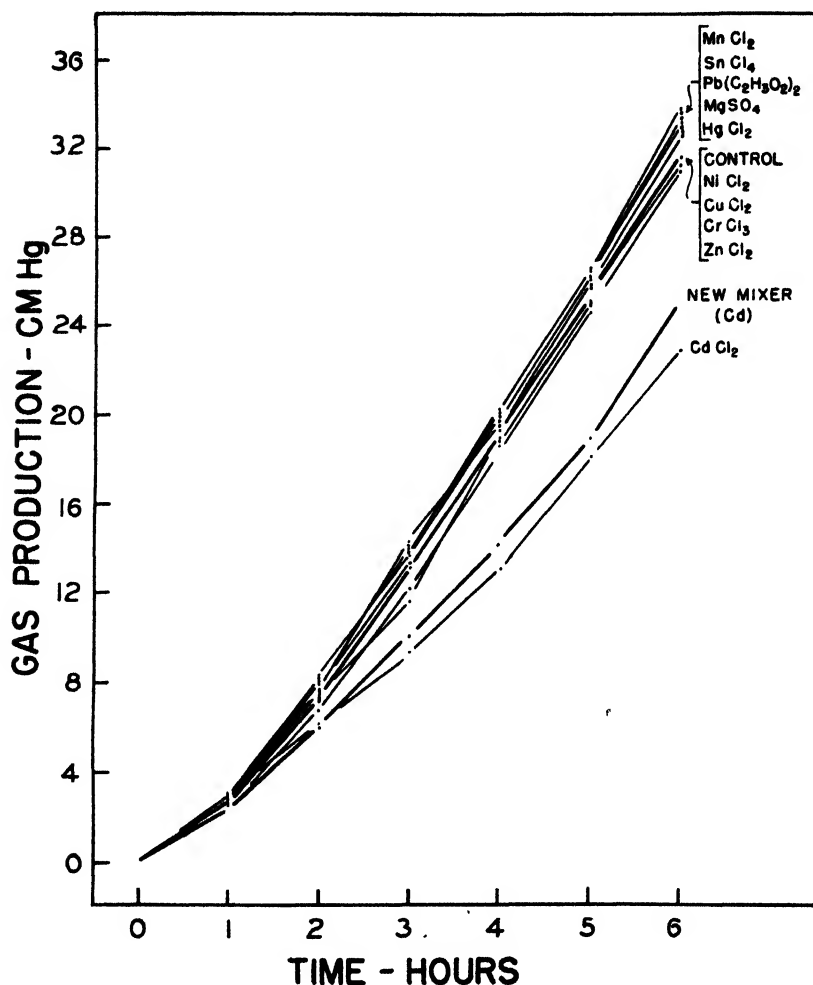


Fig. 2. Gas production of doughs containing 1 mg. concentrations of certain salts and dough mixed in new mixer (cadmium-plated planetaries).

chloride, materially below normal for mercuric chloride, and far below the control for cadmium chloride and zinc chloride.

The loaf volume and gassing data at the 100 mg. level (Table I and Fig. 4) are not only in good agreement but are of particular interest as related to the effects of lower concentrations. For example, zinc

ions affected the gas production and loaf volume in a strange manner. Concentrations of 0.001 mg. to 0.1 mg. of zinc chloride had no effect on loaf volume beyond experimental error. Addition of 1 mg. caused the loaf volume to fall slightly and gave a gassing curve (Fig. 2) that is

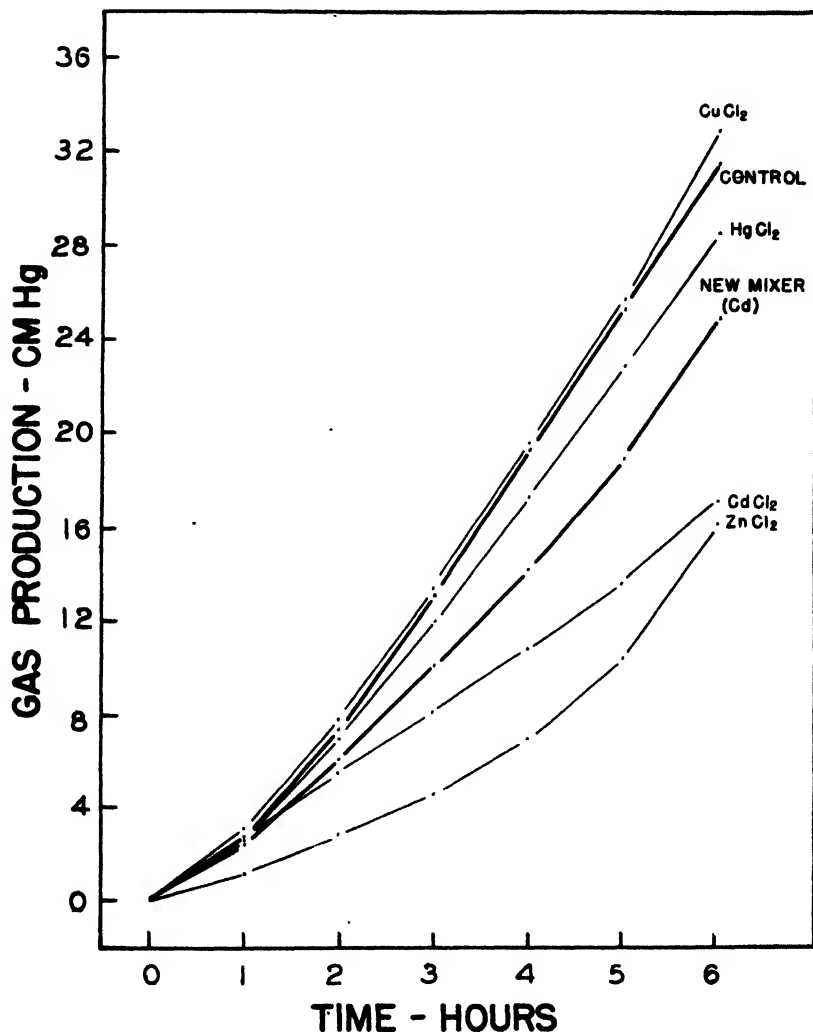


Fig. 3. Gas production of doughs containing 10 mg. concentrations of certain salts and dough mixed in new mixer (cadmium-plated planetaries).

only slightly below the control; whereas 10 mg. reduced loaf volume (Table I) to 64% normal and gas production (Fig. 3) to one-half normal. Both these values are lower than those produced by the addition of 10 mg. of cadmium chloride, the most effective inhibitor at

the lower concentrations. When 100 mg. of zinc chloride was added to the dough, however, gas production (Fig. 4) was above and loaf volume (Table I) somewhat below normal. The slightly subnormal loaf volume is assumed to be the result of excessive dough development

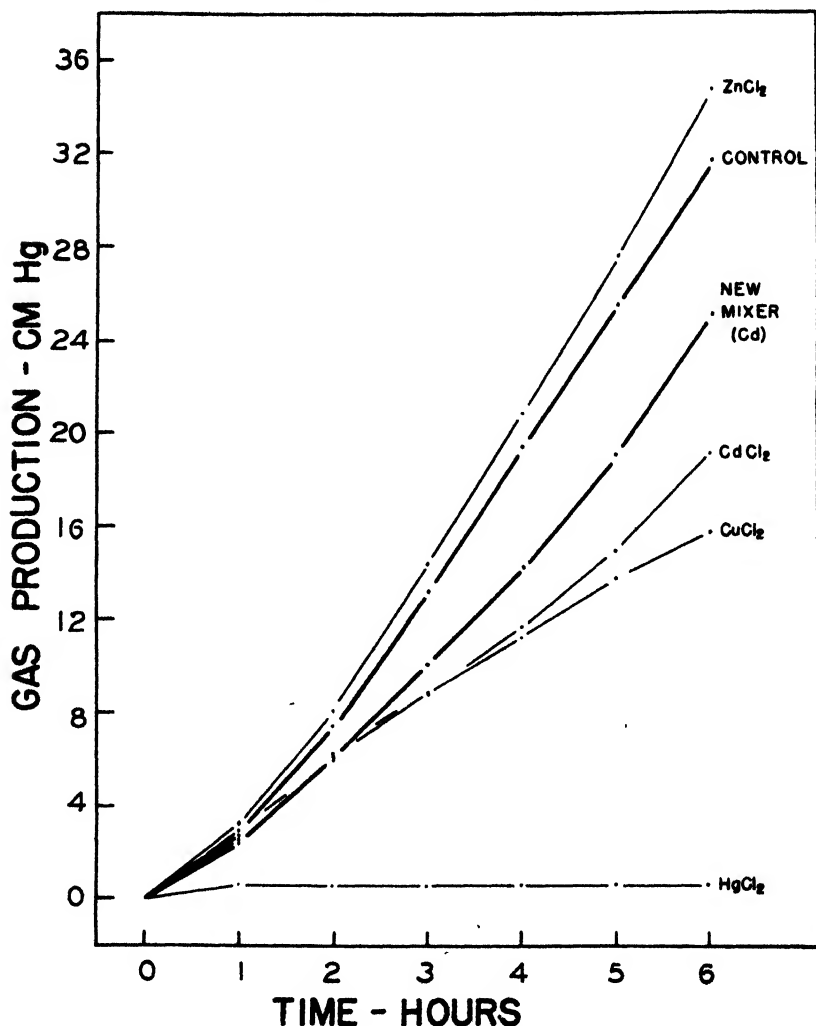


Fig. 4. Gas production of doughs containing 100 mg. concentrations of certain salts and dough mixed in new mixer (cadmium-plated planetaries).

due to above normal yeast activity. Thus at concentrations below 1 mg., the zinc ion does not act as an inhibitor; at higher concentrations it is a potent inhibitor; and at still higher concentrations yeast activity is accelerated instead of inhibited.

A large concentration of copper ions is necessary to inhibit yeast activity and reduce loaf volume. Increments of 1 mg. and 10 mg. did not affect gas production or loaf volume materially. Addition of 100 mg. cupric chloride, however, decreased gas production 50% and loaf volume 43%. These reductions are greater than those resulting from the same concentration of cadmium chloride.

Mercuric chloride, like all other salts studied excepting cadmium chloride, did not affect baking and gassing of doughs containing 1 mg. increments. A concentration of 10 mg., on the other hand, lowered gas production about 10% and loaf volume 16%; whereas 100 mg. mercuric chloride apparently stopped all enzyme activity. At the end of 72 hours the total gas produced in a dough containing 100 mg. mercuric chloride was only slightly greater than that produced after 6 hours (Fig. 4), and the dough still remained as a compact ball, indicating that proteolytic enzymes also were inactivated. The loaf volume of 235 cc. was very little larger than the volume of the dough out of the mixer. Thus zinc chloride and mercuric chloride are markedly different and each differs considerably from cadmium chloride, the only salt that inhibited yeast activity at low concentrations and whose inhibitory activity leveled off at about the same concentration where that of other salts began.

Discussion

It is well known that some enzymes involved in carbohydrate metabolism and found in wheat flour and yeast are activated whereas others are inactivated by metallic ions. For example, the activity of phosphoglucumutase is increased by Mg^{++} , Mn^{++} , or Co^{++} (6). Similarly, hexokinase, phosphatase, (enol)-phosphopyruvic acid, and carboxylase require Mg^{++} (6). β -h-Fructosidase (4) and carboxylase (5), on the other hand, are inhibited by the heavy metal ions Ag^+ , Cu^{++} , and Hg^{++} . It is necessary to refer to enzyme systems other than those involved in yeast fermentation to acknowledge previously reported effects of Cd^{++} on enzyme activity. For example, cadmium ions inactivate the amidases histidase (3) and urease (6). Previously inactivated arginase (another amidase), on the other hand, is activated by Cd^{++} (2). The effect of Cd^{++} on the activity of certain phosphatases is interesting; glycero-phosphatase is inactivated, metaphosphatase is activated, and pyro- and polyphosphatase are not affected (1). Thus, the ions of cadmium could conceivably inhibit one or more enzymes involved in yeast fermentation.

Much work to be done relates to the nature of enzyme inhibition in yeast fermentation by the salts of cadmium, zinc, copper, mercury, and others, and the specific enzymes that they inhibit. The effect of

metallic salts on *chemically leavened* baked products also should be investigated.

It is quite probable that critical parts of some commercial and household equipment have been plated with cadmium which would impair the quality of yeast-fermented products.

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EVALUATION OF DIASTATIC ACTIVITY OF MALT-CONTAINING LIQUIDS BY A MODIFIED LASCHÉ TEST ¹

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ABSTRACT

The Lasché procedure for determining amylase activity, which is based on the time required to hydrolyze a given weight of starch to the achromic point at 62.5°C., has been modified by varying the amounts of starch and diastatic liquid so that a wide range of activity can be covered. *Converting Capacities* from 0.02 to 200 can be evaluated from the equation $100 S/Lt$ where S equals the grams of starch converted to the achromic point, L is the volume (ml.) of diastatic liquid, and t the conversion time (minutes). *Acting or apparent Converting Capacity* is determined when the digestion is conducted at the pH of the diastatic liquid, whereas *maximum or potential Converting Capacities* are obtained at the optimum pH of 4.9-5.2 (at 62.5°C.). The optimum temperature for the conversion is 62.5°C. No serious thermal inactivation occurs at this temperature if the conversion time does not exceed 8 minutes. In the presence of maltose, thermal inactivation at high temperatures is less pronounced. The modified method permits a comparison of the enzyme activities of liquids from any phase of distillery operation or with the original activity of the malt which is used.

The degradation of the starch molecule by the action of malt amylases is accomplished in three major but overlapping steps, liquefaction, dextrinization, and saccharification, all of which have been

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utilized for evaluating amylase activity by measuring respectively the change in viscosity of the substrate (starch paste), the rate of dextrinization of soluble starch, and the rate of maltose production during the initial phase of the enzyme action. Within certain limitations, all three methods are suitable for evaluating the strength of solutions containing alpha- and beta-amylase in the same proportion, but none of them will give a complete picture of the various properties of a given amylase solution.

Methods measuring the dextrinization activity offer advantages in speed and simplicity. One type developed by Sandstedt, Kneen, and Blish (5) is specific for malt alpha-amylase; the other type, the Roberts method (4) and its numerous modifications by Wohlgemuth (6), Lasché (3), and Foth (2), evaluates the over-all amylase activity. The method recommended by Lasché was specially designed for evaluating distillers' malt, and, as standardized by de Becze (1), is still widely used in the United States for this purpose. The conversion temperature (62.5°C.) used in this method approximates that prevailing during the initial stages of distillery mash conversion by malt.

In the Lasché test 5 g. of soluble starch are digested in 100 ml. of aqueous solution at 62.5°C. by an infusion of 0.55 g. of ground malt. The quality of the malt is evaluated by the number of minutes necessary to reach the achromic point. This definite stage of the starch degradation is detected by the addition of 0.001 *N* iodine solution to spot plates containing samples of the digesting solution taken at intervals of 0.25 minute.

There is a great need for a simple, rapid method for evaluating amylase solutions of widely varying activity on a single scale. The experiments reported here were undertaken to develop such a method, based on the conversion time determined by the Lasché test, and to demonstrate its accuracy and use in distillery practice.

Materials and Methods

To cover the wide range of amylase concentrations encountered in distillery processes, the Lasché test has been modified to make it a more elastic method by varying the amounts of soluble starch and of amylase solutions so that the conversion time will fall between 2 to 10 (preferably 3 to 7) minutes. The selection of appropriate values for these variables permits the measurement and expression of the enzyme activity of all malt-containing liquids in the fermentation industry. The enzyme concentration is expressed by the *Converting Capacity (C)* of the liquid which is equivalent to the grams of soluble starch converted to the achromic point by 100 ml. of the amylase solution under test during one minute at 62.5°C. and pH 4.9–5.2. The enzyme

activity scale ranges from 0.02 to 200 and may be divided into three parts corresponding to the activities of "strong" (malt infusions and malt extracts), "medium" (cooker mash, day-old beer, sweet yeast mash), and "weak" (two- and three-day-old beers, sour yeast mash) enzyme solutions. For a given solution C is constant and is in direct proportion to the enzyme concentration.

The procedure for determining the *Converting Capacity* (C) of a liquid begins with the selection of the amounts of enzyme solution (L) and starch (S) to be used. The conversion time (t) is then determined, and, having the values of t , L , and S , the *Converting Capacity* (C) is calculated.

Modified Lasché Procedure

REAGENTS:

Starch solution—Merck's Soluble Starch, prepared according to Lintner, is used. For accurate results a freshly prepared starch solution must be used for each series of determinations. The required quantity of starch is placed in a beaker and mixed with one-third of the total volume of distilled water which is required to make the desired quantity of solution. The remaining water is heated to boiling in another beaker, added to the starch slurry with stirring, and the resulting mixture brought to boiling. Upon cooling, the solution is made up to the required volume.

Iodine Stock Solution, 0.1 N—13.5 g. of iodine is dissolved in a solution of 24 g. of potassium iodide in 200 ml. of distilled water and diluted with distilled water to 1 liter.

Iodine Solution, 0.001 N—This reagent should be freshly prepared (the same day when used) by diluting 1 ml. of a 0.1 N stock solution to 100 ml. with distilled water.

Sodium Hydroxide Solution, 0.1 N

Sulfuric Acid Solution, 0.1 N

Acetic Acid Solution, 15%

PROCEDURE:

1. *Preparation of the materials.* If the substance to be tested is a liquid, no preparation is required; if it is a paste or viscous syrup, such as a malt extract, it must be diluted. Solid materials, such as malt, must be pulverized and extracted with water. Solutions containing undissolved solids must be allowed to settle, centrifuged, or filtered.

The amylase solutions must not, of course, be heated and preferably should be tested immediately; however, if necessary, they may be stored in a refrigerator for a few hours.

2. *Determination of conversion time.* The amount of soluble starch may be varied from 0.5 g. to 10.0 g. (preferably 1.0 to 5.0 g.) and the amount of enzyme solution from one to 50 ml.; the total volume of the digest is always brought to 100 ml. An appropriate volume of starch solution to give the estimated amount of starch required for the material under test is pipetted into a 250 ml. electrolytic beaker which is placed in a water bath at 62.5°C. If necessary, distilled water is then added so that the total volume, after addition of the amylase solution under test, will be 100 ml. When the starch solution has attained 62.5°C., pipette in the estimated amount of diastatic liquid with thorough mixing and note the time. When more than 10 ml. of the amylase solution is required, adjust its temperature to 62.5°C. immediately before its addition to the starch solution. Place a glass tube in the digest to serve as a sampler. Add two drops of 15% acetic acid solution to each recess in two adjacent columns of a spot plate. After the digestion has proceeded 3 minutes, transfer two drops to each of the two upper recesses in the spot plate; add immediately two drops 0.001 *N* iodine solution to the left recess. Test samples every 30 seconds until the achromic point is approached when the interval is reduced to 15 seconds. The color changes from blue through purple, reddish brown, and brownish yellow to the yellow color characteristic of the iodine solution. After the achromic point has been passed, add two more drops of the standard iodine solution to each recess in the left hand column and four drops to each recess in the right hand column and determine the achromic point. The time in minutes which has elapsed after the addition of the enzyme until the starch digest assumes the constant color of the following samples is the conversion time, *t*. Cool the digest to room temperature and determine its pH.

3. *Calculation of Converting Capacity.* The *Converting Capacity* (*C*) is calculated from the equation:

$$C = \frac{100 S}{Lt}, \quad (1)$$

where *S* is the quantity of starch (dry basis) in grams present in the digest, *L* is the volume of amylase solution in ml., and *t* is the conversion time in minutes.

NOTES:

1. *Selection of the proper amount of starch and enzyme solution.* For "strong" diastatic liquids (*C* = 5.0 to 200), 5 g. of starch are used. The volume of diastatic liquid which should be taken for analysis can be estimated if its nature, and hence the approximate

value of C , is known. For example, if the *Converting Capacity* (C) of a malt infusion is 20 and the desired conversion time is 6 minutes, a suitable volume, as computed from equation (1), $L = 100 S/tC = 100 \times 5/6 \times 20 = 4.2$ ml. Accordingly 4.0 ml. is taken for analysis; this volume will be satisfactory for diastatic liquids with *Converting Capacities* between 15 and 30.

For solutions with "medium" activity ($C = 0.25$ to 5.0), 1.0 g. of starch is employed and the volume of enzyme solution is estimated in the manner already described.

For "weak" solutions ($C = 0.02$ to 0.25), 50 ml. of the solution are used and the amount of starch, S , is estimated from equation (1).

2. *Adjustment of pH.* *Converting Capacities* measured at two levels of hydrogen ion concentration are of general interest, namely: (1) at the optimum pH (4.9 to 5.2 at $62.5^\circ\text{C}.$), which gives the *maximum*, or *potential*, *Converting Capacity*; and (2) at the pH existing in the original enzyme solution which gives the *acting* or *apparent Converting Capacity*. When the pH of the digestion mixture is not optimal, the required amount of 0.1 N sodium hydroxide solution or 0.1 N sulfuric acid solution to obtain pH 4.9–5.2 is predetermined and a digestion carried out at the optimum pH. Individual adjustments of the hydrogen ion concentration, instead of using a general buffer solution, seem more appropriate because of the great variety of diastatic preparations which are to be tested.

Results and Discussion

Influence of Concentration of Reactants on Converting Capacity. Two solutions, different in origin and character, a malt infusion and a cooker mash, were employed to study the effect on *Converting Capacity* of varying the quantities of starch and enzyme solution. Three series of experiments were carried out.

In Series A, the amount of enzyme solution (L) was varied while the amount of starch (S) was kept constant. In Series B, both L and S were varied but their ratio was kept constant. In Series C, S was varied while L was kept constant. The conversion time (t) in all experiments was determined at both the natural and the optimum pH.

The results, which are summarized in Table I, show that the equation for *Converting Capacity*, $C = 100 S/Lt$, holds over a wide range of starch and enzyme concentrations. Thus, in Series A, the conversion time, or reaction velocity, is inversely proportional to the enzyme concentrations in accordance with the following equation derived from (1):

$$\frac{S}{t} = \frac{LC}{100} = kL. \quad (2)$$

TABLE I
EFFECT OF VARYING THE QUANTITY OF STARCH AND ENZYME SOLUTION
ON THE CONVERTING CAPACITY OF MALT INFUSION
AND COOKER MASH

Liquid (L)	Starch (S)	Natural pH	Conversion time (t)	Added N/10 H ₂ SO ₄	Adjusted pH	Conversion time (t)	Converting Capacity	
ml.	g.		min.	ml.		min.	C	
MALT INFUSION ¹								
Series A	3	5	5.15	10.50	0.00	5.15	10.50	15.87
	3.5	5	5.15	8.50	0.00	5.15	8.50	16.81
	5	5	5.20	6.25	0.10	5.05	6.25	16.00
	6	5	5.40	5.25	0.20	5.10	5.25	15.87
	8	5	5.55	4.75	0.30	5.10	3.75	16.67
	10	5	5.70	3.50	0.40	5.15	3.25	15.38
Series B	3	3	5.40	7.00	0.07	5.20	6.75	14.81
	4	4	5.40	6.75	0.09	5.10	6.62	15.11
	5	5	5.40	6.75	0.10	5.10	6.50	15.38
	6	6	5.40	7.00	0.13	5.15	6.75	14.81
	7	7	5.45	7.00	0.16	5.10	6.87	14.56
	8	8	5.45	7.00	0.21	5.10	6.75	14.81
Series C	3	0.5	5.35	1.25	0.05	5.10	1.25	13.40
	3	1	5.25	2.50	0.05	5.20	2.25	14.81
	3	2	5.20	4.50	0.00	5.20	4.50	14.81
	3	3	5.10	6.50	0.00	5.10	6.50	15.38
	3	4	5.10	9.00	0.00	5.10	9.00	14.81
	3	5	4.90	11.50	0.00	4.90	11.50	14.49
DISTILLERS' CORN COOKER MASH ²								
Series A	10	1	6.40	14.00	0.45	5.30	12.50	0.80
	15	1	6.35	9.00	0.60	5.30	8.00	0.83
	20	1	6.30	7.00	0.90	5.25	6.00	0.83
	30	1	6.30	4.50	1.30	5.35	4.00	0.83
	40	1	6.40	3.50	1.70	5.40	3.00	0.83
	50	1	6.40	2.75	2.00	5.25	2.50	0.80
Series B	5	0.25	6.60	8.50	0.30	5.20	6.50	0.77
	10	0.50	6.50	8.00	0.55	5.25	6.00	0.83
	20	1.00	6.30	7.00	0.90	5.25	6.25	0.80
	30	1.50	6.15	7.00	1.20	5.20	6.00	0.83
	40	2.00	6.30	7.00	1.60	5.25	6.00	0.83
	50	2.50	6.15	7.00	2.00	5.30	6.25	0.80
Series C	20	0.2	6.20	2.00	1.00	5.25	1.50	0.67
	20	0.4	6.20	3.00	1.00	5.35	2.50	0.80
	20	0.6	6.30	5.00	1.00	5.25	3.75	0.80
	20	0.8	6.35	6.00	1.10	5.20	5.00	0.80
	20	1.0	6.30	7.00	1.20	5.20	6.25	0.80
	20	1.2	6.00	8.50	1.30	5.20	7.25	0.83

¹ Prepared by extracting 100 g. ground malt with 400 ml. distilled water for one hour at 30°C. A different distillers' malt was used for each series.

² The same liquid was used for all three series.

From this equation, it is evident that when the $\frac{\text{starch}}{\text{liquid}}$ ratio is kept constant, the conversion time must be constant for a given enzyme solution.

$$t = \frac{S}{kL} = K. \quad (3)$$

Within certain limits, the t values determined at optimum pH in Series B are constant.

Since reaction velocity varies directly with the enzyme concentrations, the conversion time t will vary directly with the amount of starch (S) when enzyme concentration is constant.

$$t = K_2 S. \quad (4)$$

The experimental values for Series C indicate that the changes in conversion time bear a linear relation to changes in the amount of starch.

The data, therefore, demonstrate that the *Converting Capacity* of a given amylase solution determined according to the prescribed method is independent of variations in the volume of enzyme solution or quantity of starch over wide limits and prove the validity of equation (1) on which the determination of the *Converting Capacity* (C) is based.

The accuracy of the method is affected by such factors as error in reading the end point, error in adjusting the pH to the optimum, and the effect of thermal destruction of the enzyme during the conversion. With good technique it is possible to determine t to the nearest 0.25 minute.

Effect of pH. Experiments were conducted to determine the optimum pH for *Converting Capacity* at 62.5°C., and to ascertain the effects of variations in starch and enzyme concentration on the optimum pH.

The conversion times of amylase solutions were determined at pH values from about 3.7 to 8.0 and the *Converting Capacities* calculated. In addition, the enzyme concentration, in the digest, volume of enzyme solution \times *Converting Capacity* (LC), and the ratio of starch to enzyme volume $\frac{100 S}{L}$ were calculated. The determinations were grouped in three series:

In the first, both the amount of starch (S) and enzyme solution (L) were kept constant and the pH varied from 3.8 to 8.2.

In the second, the volume of enzyme solution (L) was kept constant (2 ml.) and the starch was varied between 0.5 and 10 g. to give starch to enzyme volume ratios of 500, 250, 125, 50, and

25. With each amount of starch, eight determinations were made at pH values from 4.2 to 5.6, about 0.2 apart.

In the third series, the quantity of starch (*S*) was kept constant and the volume of enzyme solution (*L*) was varied (5, 10, 15, and 20 ml.); for each volume, determinations were made at pH values ranging from 4.2 to 6.0.

The results of the first series, summarized in Table II, show that optimum activity of the malt infusion is obtained between about pH 5.0 and 5.4.

The second series of experiments established that changes in the amount of starch (0.5 to 10 g.) with a constant volume of enzyme solution did not change the optimum pH.

TABLE II

EFFECT OF PH ON THE CONVERTING CAPACITY OF MALT INFUSION AT 62.5°C.

pH	Converting Capacity (C)	pH	Converting Capacity (C)	pH	Converting Capacity (C)	pH	Converting Capacity (C)
3.80	0.00	5.00	19.20	5.30	19.30	6.40	9.72
4.10	1.18	5.00	20.00	5.40	19.30	6.75	7.70
4.25	2.73	5.10	20.00	5.45	19.30	6.75	7.28
4.35	13.47	5.15	20.02	5.70	18.13	7.00	5.73
4.55	14.20	5.15	19.30	5.90	14.87	7.20	3.16
4.70	18.13	5.25	19.30	6.10	14.56	7.90	0.94
4.85	18.71	5.30	19.00	6.20	13.20	8.15	0.91

The third series of experiments indicated that there is a slight shift in the optimum pH with a change in the volume of any given enzyme solution. Additional experiments with different solutions indicated that the optimum pH is a function of the enzyme concentration as expressed by the *enzyme volume* \times *Converting Capacity* (*LC*). However, the *Converting Capacity* (*C*) only changed about 5% at a pH of 5.0 to 5.2 with variations in *LC* values from 5 to 225.

The optimum pH for different *LC* values was found to be:

<i>LC</i>	pH
225	4.75-4.95
150	4.85-5.05
75	5.00-5.20
24	5.25-5.45
5	5.65-5.75

The enzyme concentration in the digest should be selected, if possible, to fall within *LC* values of 50 to 150 when using the pH optimum of 4.9 to 5.2. When log *LC* is plotted against the optimum pH the result is a straight line satisfying the equation:

$$\text{pH}(\text{optimum}) = -0.515 \log LC + 6.06 \quad (5)$$

Effect of Temperature. The effect of digestion temperature on the *Converting Capacity* of a malt infusion was studied at 12 temperatures between 20° and 80°C. at pH 4.9 to 5.2 Above 60°C. two determinations, and at 70° and 80°C. three determinations, were made with different enzyme concentrations to demonstrate the effect of thermal inactivation on the determination. The data reported in Table III demonstrate that the activity increases up to 62.5°C. Between 30° and 50°C., the activity increases about 1.7 times per 10°C.

TABLE III
INFLUENCE OF DIGESTION TEMPERATURE ON CONVERTING CAPACITY
(pH = 4.9-5.2)

Digestion temp.	Enzyme solution	Starch	Conversion time	Converting Capacity
°C.	ml	g.	min.	C
20.0	30.0	2.5	25.00	0.33
30.0	6.0	5.0	16.00	5.42
40.0	5.0	5.0	10.50	9.52
50.0	4.0	5.0	7.50	16.66
55.0	3.0	5.0	8.00	20.82
60.0	2.0	5.0	10.50	23.80
62.5	2.0	5.0	10.00	25.00
62.5	10.0	5.0	2.00	25.00
65.0	2.0	5.0	10.00	25.00
65.0	10.0	5.0	2.00	25.00
67.5	2.0	5.0	11.50	21.73
67.5	10.0	5.0	2.00	25.00
70.0	2.0	5.0	18.00	13.88
70.0	3.0	5.0	8.50	19.60
70.0	10.0	5.0	2.00	25.00
72.5	2.0	5.0	30.00	8.33
72.5	5.0	5.0	5.00	20.00
75.0	5.0	5.0	25.00	4.00
75.0	10.0	5.0	2.50	20.00
77.5	5.0	5.0	--	0.00
77.5	10.0	5.0	5.00	10.00
80.0	5.0	5.0	--	0.00
80.0	10.0	5.0	32.00	1.56
80.0	20.0	5.0	1.50	16.66

At the optimum temperature of 62.5°C. (and also at 65°C.) the value of C is the same for 2 and 10 minutes conversion time so that thermal inactivation was not evident in this length of time, possibly due to the protective activity of substances present in the digest. The first evidence of thermal inactivation is seen at 67.5°C., but even at 70°C. full activity was obtained when the conversion time was decreased to 2 minutes. These experiments demonstrate that no serious thermal destruction occurs at 62.5°C. if the conversion time is maintained between 4 to 8 minutes.

Thermal Destruction of the Enzyme and Its Inhibition. The thermal destruction of the enzyme at 62.5°C. was studied in four series of ex-

periments, where the *Converting Capacities* of four different enzyme solutions were determined after exposing them to a temperature of 62.5°C. for various lengths of time just before adding to the starch solution. The four amylase solutions were: (1) a malt infusion prepared by extracting ground malt with water, (2) a cooker mash, (3) a malt infusion prepared by extracting malt with previously boiled cooker mash, and (4) a malt infusion prepared in a similar fashion to (1) but enriched with 10% maltose.

Before hydrolysis the pH was adjusted to the optimum (4.9 to 5.2) in each case, and the temperature of the amylase solutions was raised to 62.5°C. in less than 2.5 minutes prior to the heat treatment. The

TABLE IV
EFFECT OF TIME ON AMYLASE DESTRUCTION AT 62.5°C.
IN VARIOUS EXTRACTS
(pH = 4.9-5.2)

Exposure time	Aqueous malt extract ¹		Cooker mash ²		Malt extract with cooker mash ³		Malt extract with maltose added ⁴	
	Converting Capacity	Activity	Converting Capacity	Activity	Converting Capacity	Activity	Converting Capacity	Activity
<i>min.</i>	<i>C</i>	<i>%</i>	<i>C</i>	<i>%</i>	<i>C</i>	<i>%</i>	<i>C</i>	<i>%</i>
0	13.60	100	1.66	100	24.85	100	12.99	100
5	11.60	84	1.62	97	23.81	96	12.99	100
10	10.35	76	1.55	93	22.86	92	12.42	96
15	9.23	68	1.51	90	19.70	79	12.42	96
30	7.21	53	1.28	77	14.81	60	11.91	92
45	6.10	45	1.13	68	12.50	50	10.58	81
60	5.43	40	1.00	60	11.90	48	10.20	78
90	4.46	33	0.57	34	10.02	40	8.93	69
100	4.35	32	0.41	25	9.15	36	7.54	57

¹ 100 g. ground malt extracted in 400 ml. H₂O at 30°C. Balling = 5.05, pH = 5.85.

² Straight cooker mash. Balling = 18.0, pH = 5.30.

³ Same as ¹ except malt was extracted in boiled cooker mash instead of water. Balling = 28.5, pH = 5.50.

⁴ Malt infusion prepared as in ¹ with 10% added maltose. Balling = 15.13, pH = 5.70.

starch to enzyme ratio was selected to yield a conversion time between 4 and 8 minutes. The conversion times and the activities retained after the heat treatments, expressed in percentage of the starting activities, are given in Table IV. The delay in heat destruction in series (2), (3), and (4), as compared with series (1), is attributed to the protective action of the maltose.

Use of the Converting Capacity in Distillery Control. Measurement of the enzyme activity of diastatic liquids according to the proposed method with its broad scale is particularly useful in distillery control. The enzyme activities of liquids from any production phase can be compared with each other or directly with the original activity of the

TABLE V
CHANGES IN CONVERTING CAPACITY OF COOKER MASH ON THE WAY
FROM THE COOKER TO FERMENTER

Converting Capacity in cooker	Time between samples	Converting Capacity in fermenter	Activity lost
<i>C</i>	<i>min.</i>	<i>C</i>	<i>%</i>
1.25	25	1.19	4.0
0.89	20	0.81	9.0
0.89	20	0.89	0.0
0.96	20	0.74	23.0
1.19	15	1.09	8.0
1.33	20	1.07	19.0
1.27	15	1.18	7.0
1.07	40	0.91	15.0
1.57	30	1.40	10.0

malt which is used. To demonstrate this, *Converting Capacity* for two groups of samples collected from various production phases was determined at the optimum pH (4.9 to 5.2).

In the first group, samples were taken from nine freshly converted cooker mashes coming from the cooker and also from the same mashes after they passed the coolers. The temperature of the mash was 62°C. before entering the cooler and about 25°C. on leaving it. The time which elapsed between each pair of samples, their activity, and the per cent activity lost are given in Table V.

In the second group of samples the loss of amylase activity in beer from the time of inoculation until the end of the fermentation was followed in four separate fermenters. The *Converting Capacities* of samples taken at different intervals and the losses in activity are sum-

TABLE VI
CHANGES IN CONVERTING CAPACITY OF BEER DURING FERMENTATION

Age of beer	Fermenter No. 1		Fermenter No. 2		Fermenter No. 3		Fermenter No. 4	
	Convert- ing Ca- pacity	Loss	Convert- ing Ca- pacity	Loss	Convert- ing Ca- pacity	Loss	Convert- ing Ca- pacity	Loss
<i>hr.</i>	<i>C</i>	<i>%</i>	<i>C</i>	<i>%</i>	<i>C</i>	<i>%</i>	<i>C</i>	<i>%</i>
0	1.07	0	1.18	0	0.94	0	1.40	0
5	0.81	24	0.77	35	0.79	16	1.10	22
10	0.71	34	0.75	37	0.54	43	0.70	30
20	0.50	53	0.73	38	0.42	56	0.55	45
24	0.32	70	0.71	40	0.38	60	0.36	64
28	0.21	80	0.63	47	0.21	78	0.31	69
44	0.07	94	0.11	90	0.06	94	0.12	88
48	0.02	98	0.08	93	0.05	95	0.05	95
52	0.02	98	0.06	95	0.04	96	0.04	96
68	0.00	100	0.04	96	0.04	96	0.02	98
72	0.00	100	0.02	98	0.03	97	0.00	100

marized in Table VI. The extensive reduction in amylase activity is the cause of the slow conversion rate during the later phases of fermentation.

The *Converting Capacity* of the beer at any given age indicates the activity of the enzyme under the conditions existing in the fermenter. This may be determined at the pH and temperature of the beer or, in an accelerated test, at the pH of the beer but at 62.5°C. Determinations of the *acting Converting Capacity* parallel with determinations of the *maximum Converting Capacity* reveal that the change in pH which occurs in the fermenter inhibits amylase activity.

In a water mash, after 30 to 34 hours fermentation, the acids produced may decrease the pH to 4.0 or below, at which pH the activity of the amylase is negligible. Because of this, conversion and fermentation cease in spite of the presence of considerable amount of amylase. When stillage is added to the beer, the pH remains at higher values due to the buffering effect, permitting a more complete conversion and fermentation.

In industrial control the determination of both *maximum* and *acting Converting Capacity* has proven to be a useful tool.

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FEEDING TESTS WITH CHLORINE DIOXIDE-TREATED FLOUR¹

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ABSTRACT

Dogs have been fed flour rations treated with as much as 4 g. chlorine dioxide per hundredweight of flour for as long as 13 weeks and have remained in a healthy condition without any indication of "running fits." Rabbits and monkeys grew well and maintained themselves on chlorine dioxide-treated rations for periods of 6 weeks and 5½ months, respectively. Blood analyses and histological examination of these animals were all within normal limits. Rats grew equally well on untreated or chlorine dioxide-treated flour and bread rations.

Thirteen human subjects were fed normal diets supplemented with large amounts of wheat flour and wheat gluten treated with high levels of chlorine dioxide. Electroencephalograms, neurological examinations, blood analyses, and urinalyses were all within normal limits for each subject at the end of the 6-week experimental periods.

It was first reported by Mellanby (1) that dogs developed "running fits" when fed rations containing wheat flour treated with nitrogen trichloride. Newell *et al.* (2) reported that cats, ferrets, and mink were also susceptible to the nitrogen trichloride toxicity. Monkeys did not develop running fits when fed nitrogen trichloride-treated rations but abnormal brain wave patterns were reported.

In November, 1947, the Food and Nutrition Board of the National Research Council published the following statements concerning the use of nitrogen trichloride: ". . . in view of the susceptibility of several mammalian species there is definite risk of injury to human beings. . . . Alternate processes of flour treatment, especially with chlorine or chlorine dioxide, should be thoroughly explored to make certain that flour so treated is free from toxic effects." With these recommendations in mind, investigation of chlorine dioxide was undertaken. Studies were made on animals known to be susceptible to nitrogen trichloride toxicity and on human beings.

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Materials and Methods

Mongrel dogs ranging in age from 3 to 6 months were used in these experiments. Each animal was dewormed, dusted with DDT (4,4'-dichlorodiphenyltrichloroethane), protected against distemper with injections of canine antidistemper serum, and maintained on a purified basal ration for a week or more before being placed on experiment.

The ration used for the dogs had the following composition: wheat flour, 84; crude casein, 5; corn oil, 5; salts IV, 4; and whole liver powder, 2. Oral supplements of 0.75 mg. thiamine, 0.75 mg. riboflavin, 0.5 mg. pyridoxine, 0.25 mg. pantothenic acid, 1 mg. nicotinic acid, and 70 mg. choline per kilogram of body weight and 6 drops of halibut liver oil were administered twice weekly. Three dogs were fed the above ration containing untreated flour for 12 weeks; two were fed flour treated with 0.5 g. of chlorine dioxide per hundredweight for 6 and 12 weeks; one was fed flour treated with 1.0 g. of chlorine dioxide per hundredweight for 6 weeks; three were fed flour treated with 2 g. of chlorine dioxide per hundredweight for periods of 11 to 13 weeks; two were fed flour treated with 4 g. of chlorine dioxide per hundredweight for 12 weeks. Consumption records were kept for each animal so that an accurate evaluation of the particular flour under test could be made if toxicity developed.

TABLE I
RESULTS OF FEEDING RABBITS RATIONS CONTAINING CHLORINE
DIOXIDE-TREATED FLOUR

RATION COMPOSITION (per 100 g.)											
Flour	60 g.	Pyridoxine	1.0 mg.								
Gum arabic	12 g.	Nicotinic acid	10.0 mg.								
Casein	15 g.	Pantothenic acid	3.0 mg.								
Brewers' yeast	5 g.	Inositol	10.0 mg.								
Fortified		p-Aminobenzoic									
soybean oil ¹	4 g.	acid	10.0 mg.								
Salts IV ²	4 g.	Biotin	0.04 mg.								
Thiamine	1 mg.	Folic acid	0.03 mg.								
Riboflavin	1.4 mg.	Choline	300.00 mg.								
GROWTH RESULTS											
Flour... ..	Untreated					4 g. ClO ₂ /cwt.					
Rabbit	1	2	3	4	5	7	8	9	10	11	12
G. gained in 6 weeks	725	410	895	1085	770	1095	1055	1380	740	1110	1090
Abnormalities	None	None	None	None	None	None	None	None	None	None	None

¹ The following vitamins were dissolved in 4 g. of soybean oil: 12 mg. β -carotene, 0.008 mg. D₃, 0.4 mg. K (Menadione), 120 mg. E.

² J. Biol. Chem. 109: 657 (1935).

Weanling male and female New Zealand White rabbits were obtained from Old Orchard Rabbit Farm, Madison, Wisconsin, and placed on rations containing 60% flour for a period of 6 weeks. The composition of these rations is shown in Table I. Five rabbits were fed rations containing untreated flour, and six others were fed rations containing flour treated with 4 g. of chlorine dioxide per hundredweight. At periodic intervals blood samples were obtained from each animal and the usual analyses (serum protein, red blood cell count, white

TABLE II
RESULTS OF FEEDING MONKEYS RATIONS CONTAINING CHLORINE
DIOXIDE-TREATED FLOUR ¹

	Bread made from chlorine dioxide-treated (1 g./cwt.) flour		Bread made from untreated and chlorine dioxide-treated (4 g./cwt.) flour	
	%		%	
Flour	78		68	
Casein	5		15	
Corn oil	5		5	
Salts IV	4		4	
Whole liver powder	2		2	
Yeast	3		3	
Sucrose	3		3	

MAINTENANCE RESULTS									
Flour... ..	Untreated			1 g. ClO ₂ /cwt.		4 g. ClO ₂ /cwt.			
Monkey	500	503	505	C	D	501	502	504	506
Months on expt.	5½	5½	5½	1½	1½	5½	5½	3	3
G. change in weight	+725	+930	+1880	+175	+865	+1880	+2085	-775	+1020
Abnormalities	None	None	None	None	None	None	None	²	None

¹ Vitamin supplements per 100 g. of dried, ground bread: 25 mg. ascorbic acid, 1 mg. thiamine, 1 mg. riboflavin, 1 mg. pyridoxine, 5 mg. nicotinic acid, 20 µg. biotin, 3 mg. pantothenic acid, 25 mg. choline, 15 mg. inositol, 50 mg. p-aminobenzoic acid, 100 µg. folic acid. Two drops of halibut liver oil. were administered to each monkey weekly.

² Cause of weight loss undetermined.

blood cell count, differential white cell count, and hemoglobin) were made. At the end of the test period the animals were sacrificed, examined post mortem, and samples from the central nervous system, all vital organs, and representative tissues were removed and studied histologically.

Nine monkeys were fed bread rations made from untreated flour and chlorine dioxide-treated flour for periods of 6 weeks to 5½ months. The composition of the rations and the results of these tests are given in Table II. Three monkeys were fed untreated bread rations for 5½ months; two monkeys were fed bread made from flour treated with 1 g. of chlorine dioxide per hundredweight for a period of 6 weeks; four

monkeys were fed bread made from flour treated with 4 g. of chlorine dioxide per hundredweight for as long as 5½ months. Blood samples were obtained at 5-week intervals from the monkeys on the long-term tests. The blood was analyzed for serum protein, red blood cell count, white blood cell count, differential white cell count, and hemoglobin. At the end of the test period the animals were sacrificed, examined post mortem, and samples from the central nervous system, all vital organs, and representative tissues were removed and studied histologically.

Weanling male rats of the Sprague-Dawley strain were fed rations containing whole wheat flours, white flours, and bread baked from the

TABLE III
RESULTS OF FEEDING RATS RATIONS CONTAINING
CHLORINE DIOXIDE-TREATED FLOUR¹

Group	I	II	III	IV	V	VI
Flour	Untreated whole wheat flour	1.25 g. chlorine dioxide per cwt. of whole wheat flour	Untreated white flour	4 g. chlorine dioxide per cwt. of white flour	Bread from flour, Group III	Bread from flour, Group IV
	%	%	%	%	%	%
Flour	84	84	81	81	78	78
Casein	5	5	5	5	5	5
Corn oil	5	5	5	5	5	5
Salts IV	4	4	4	4	4	4
Liver powder	2	2	2	2	2	2
Yeast	—	—	—	—	3	3
Sucrose	—	—	3	3	3	3
Rats per group	6	6	20	20	20	20
Avg. wt. at end of 5 weeks (g.)	216	218	174	184	187	177
Abnormalities	None	None	None	None	None	None

¹ Vitamin supplements of: 2 mg. thiamine, 1 mg. riboflavin, 3 mg. pyridoxine, 20 mg. pantothenic acid, 1 g. choline were mixed into each kg. of flour ration and into each kg. of dried, ground bread ration. One drop of halibut liver oil administered orally per rat per week.

white flours for 5-week periods. The whole wheat flours were untreated or treated with 1.25 g. of chlorine dioxide per hundredweight; the white flours were untreated or treated with 4 g. of chlorine dioxide per hundredweight. Composition of the rations fed the rats is shown in Table III.

Thirteen healthy volunteer male and female human beings were fed normal diets supplemented daily with 55 g. of wheat protein treated with high amounts of chlorine dioxide for periods of 6 weeks. These volunteers ranged in age from 19–25 and were students at the university. Each subject received a neurological examination before being

fed the test diet, midway through the experiment, and at the end of the test period. Examination was made of the skull, spine, cranial nerves, motor system, sensory system, autonomic nervous system, blood pressure, and mental status of each subject. Complete blood counts, urinalyses, and serum protein determinations were also made. Electroencephalograms (measurements of brain wave patterns) were taken at 3- to 4-day intervals on all subjects during the test period. Each subject had six to eight control electroencephalograms taken before being started on the test diet. Food supplements were weighed and the net daily consumption of the supplement was tabulated by hospital dietitians. Flour treated with 4 g. of chlorine dioxide per hundredweight and wheat gluten treated with 20 g. of chlorine dioxide per hundredweight were included in daily supplements of cereal and muffins. The wheat products used received four to eight times the average commercial treatment of 0.5 g. of chlorine dioxide per hundredweight of flour, and were fed in considerably higher amounts than are found in the average daily diet.

Results and Discussion

It is evident from the results in Table IV that the three dogs which received untreated flour and the eight dogs which received flour treated with varying levels of chlorine dioxide showed neither running fits nor

TABLE IV
RESULTS OF FEEDING DOGS RATIONS CONTAINING
CHLORINE DIOXIDE-TREATED FLOUR

Dog no.	Flour treatment per cwt.	Days on experiment	General condition	Abnormalities
236, 248, 251	Untreated	84	Good	None
241	0.5 g. ClO ₂	42	Good	None
246	0.5 g. ClO ₂	84	Good	None
27	1.0 g. ClO ₂	42	Good	None
250	2.0 g. ClO ₂	77	Good	None
30 ¹	2.0 g. ClO ₂	84	Good	None
244	2.0 g. ClO ₂	91	Good	None
293 ¹	4.0 g. ClO ₂	84	Good	None
299 ¹	4.0 g. ClO ₂	84	Good	None

¹ Normal electroencephalograms obtained at end of test period.

any other abnormalities which could be attributed to the chlorine dioxide treatment. All animals were in a healthy condition and showed steady weight gains throughout the experiment. Electroencephalograms were taken on dogs 30, 293, and 299 at the beginning and at the end of the test period. Although flour treatment was at the high level of 4 g. of chlorine dioxide per hundredweight and the test ration was fed for 84 days (12 weeks), no abnormalities were observed

in the electroencephalograms of any of the dogs. Previous experience, Newell *et al.* (2), indicated that flour treated with low commercial levels of nitrogen trichloride (1 g./cwt.), and fed at high levels of the ration, produced running fits in dogs in at least 45 days. It was felt that any toxic condition which might develop in dogs as a result of chlorine dioxide treatment of flour would occur within a 12-week period.

Growth results obtained with rabbits fed rations containing 60% of flour for 6 weeks are summarized in Table I. The semipurified rations fed these animals sustained good growth when compared with natural rations fed rabbits in this laboratory. Rabbits fed nitrogen trichloride-treated flour rations (20 g./cwt.) readily develop running fits in 5 to 10 days and die or are permanently paralyzed. Rabbits fed the untreated or chlorine dioxide-treated flour were in a healthy condition throughout the experiment. Those fed the treated flour rations grew better than those fed the untreated flour rations, but no significance was attributed to this difference. Blood analyses and histological examination of the tissue samples were all within normal limits.

Results obtained with monkeys fed bread made from untreated and chlorine dioxide-treated flour are summarized in Table II. One animal suspected of having incipient tuberculosis was sacrificed after being on experiment 3 months. However, post mortem examination of this animal showed no abnormal conditions and histological examination of the tissues revealed no abnormalities. All other monkeys used in this work grew and maintained themselves throughout the test period. Blood analyses were normal; post mortem examinations showed no pathological conditions; histological examination of the tissues revealed no abnormalities.

Growth results of rats fed flour and bread rations are shown in Table III. Rats fed the untreated and chlorine dioxide-treated whole wheat rations grew well during the 5-week test period. Rats fed flour or bread rations made from treated white flour showed growth responses similar to those of rats fed flour or bread rations made from untreated white flour. There was no evidence of toxicity from any of the flours, even when the 4 g. of chlorine dioxide per hundredweight treatment was used.

All subjects fed the chlorine dioxide-treated food supplements for 6-week periods remained in healthy condition throughout the experiment. Electroencephalograms were normal for each subject and showed no changes from the normal patterns obtained during the control period. Most subjects gained weight while on the test diet as a result of the high protein intake. Blood, urine, and neurological

examinations made on each patient were normal at the end of the experiment.

Acknowledgments

We are indebted to Dr. H. K. Parker, to Meade C. Harris, and to Karl L. Fortmann, of the Wallace and Tiernan Company, for preparing the chlorine dioxide-treated food materials.

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HYGROSCOPIC EQUILIBRIA OF WHOLE KERNEL CORN¹

S. F. BROCKINGTON,² H. C. DORIN,² and H. K. HOWERTON³

ABSTRACT

The relative humidities of air in equilibrium with shelled yellow corn conditioned to moisture contents between 9.4 and 22.6% were determined at $80^{\circ} \pm 0.2^{\circ}\text{F.}$ with a new type of electric hygrometer. Moisture determinations were carried out with the Brown-Duvel apparatus as well as by a two-stage vacuum oven technique in which the second stage involved drying at 135°C. in a vacuum oven for 5 hours. The critical moisture content of shelled, yellow corn for safe storage, namely that in equilibrium with a relative humidity of 75%, was found to be $13.8 \pm 0.2\%$ by the Brown-Duvel method and $14.7 \pm 0.1\%$ by the two-stage vacuum oven technique.

It is well recognized that spoilage in stored grain depends largely on its moisture content. Above a certain critical moisture range, marked acceleration in the respiratory rate occurs and heating tendencies appear. Milner and Geddes (6), Gilman and Semeniuk (5), and others have shown that this acceleration of respiration and the accompanying increase in fat acidity of various grains occurs at moisture contents in equilibrium with a relative humidity of approximately 75%, which is about the lowest humidity at which the common storage molds (species of *Aspergillus* and *Penicillium*) present on and in the grain will grow. It is therefore of prime importance in the safe storage of corn to know the moisture content at which the grain is in equilibrium with 75% relative humidity.

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³ The American Instrument Company, Silver Spring, Maryland.

Many determinations of the hygroscopic equilibria of various grains have been made. Coleman and Fellows (2) passed air at various humidities over previously dried grain and determined the moisture contents after equilibrium was attained. Their moisture data were computed on the dry basis and must be multiplied by the factor $100/100 + M$, where M is the moisture content on a dry basis to convert them to the customary values on the wet basis. Oxley (7) has recently discussed hygroscopicity data obtained on cereal grains by several workers. Although there is lack of agreement concerning the equilibrium moisture values for given humidities, all investigators obtained curves of the sigmoid type. The relative humidity increases rather sharply with increases in moisture between 4% and 16% and small increases in moisture will therefore cause wide increases in ambient relative humidity. Cereal grains containing between 14% and 15% moisture are in approximate equilibrium with 75% relative humidity. Above 15% moisture, the humidity increases more slowly with increase in moisture and the curves tend to approach an asymptotic value at about 95% relative humidity.

Oxley (7) suggests that it would be better to measure ambient humidity of the surrounding air in grain directly, after previously wetting, rather than to pass air at different humidities over dried grain in order to study hygroscopic equilibria. The sigmoid shape of the curves suggests that the absorption of water by grain and vapor pressure of moist grain follow hysteresis patterns.

The present study is concerned with the determination of the ambient relative humidity of whole kernel yellow corn, conditioned to various moisture contents, by an electric hygrometer similar to that described by Dunmore (4). This instrument was found to be extremely sensitive and afforded a relatively simple method for determining the relative humidity in a small space.

Materials and Methods

Apparatus. The electric hygrometer, consisting essentially of a humidity-sensing element and a microammeter with necessary electrical connections, was made and calibrated by the American Instrument Company. The composite sensing element, hereafter designated 4TH2, was constructed from four single elements. The operation of the element is based on the ability of a hygroscopic film to change its electrical resistance quickly with small changes in moisture content of the air surrounding it. The resistance was measured in microamperes of alternating current flowing through the element as shown by the electrical indicator. A single element is shown in Fig. 1.

By electrically coupling four single elements through various

resistors, the completed 4TH2 element when connected to the indicator gave readings which were proportional to the relative humidity. A small resistance thermometer was incorporated in the 4TH2 element as a temperature-measuring device to determine the necessary corrections in humidity readings for variations in temperature. The instrument was calibrated approximately one week before the experiments on corn were made, in a controlled atmosphere. Wet- and

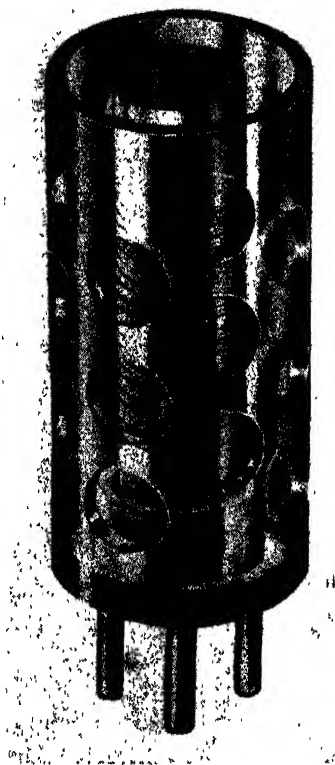


Fig. 1. Type H-2 humidity-sensing element.

dry-bulb temperatures were measured by mercury-in-glass thermometers to an accuracy of $\pm 0.5^{\circ}\text{F}$. Depression of the wet bulb for various humidities was extrapolated from the psychrometric tables published by the Weather Bureau, U. S. Department of Commerce. The calibration curves for three temperatures are shown in Fig. 2 and are accurate to within 0.5% relative humidity.

The corn was placed in a quart jar as shown in Fig. 3. The 4TH2 element was mounted on the cover of the jar by means of an amphenol

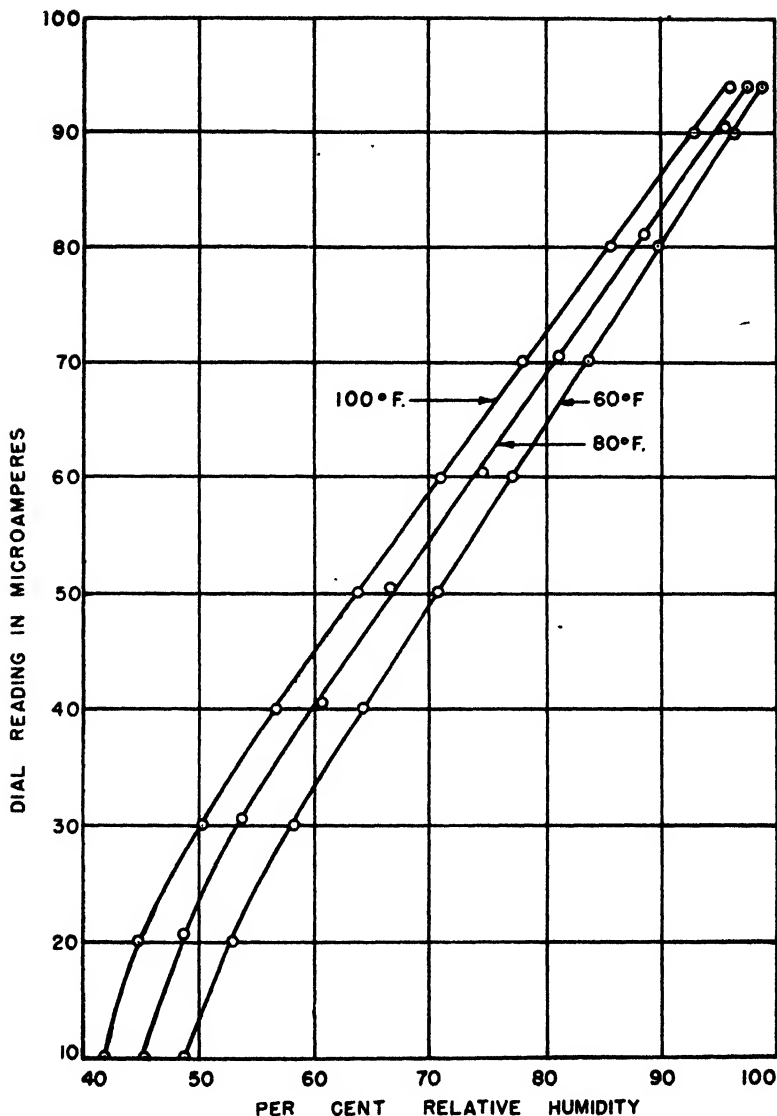


Fig. 2. Calibration of humidity-sensing element.

connector which served as a receptacle for the leads and as a means of obtaining an effective seal between the cover and the element. A fine mesh brass screen was placed around the element for protection.

The corn used in these experiments was naturally dry No. 2 yellow corn of the 1947 crop which was approximately 7 months old. It was cleaned free of debris and broken kernels in the laboratory.

Sufficient tap water was added to 1-kg. samples to increase the water content to the desired value. The samples were mixed thoroughly and allowed to stand 16 to 24 hours to equalize the moisture throughout, before humidity determinations were made.

Humidity Determination. About 500 g. of the moistened grain were placed in the container, the sensing element completely submerged in the grain, the cover screwed down, and the jar placed in a water bath at $80^{\circ} \pm 0.2^{\circ}\text{F.}$, with the water level in relation to the jar adjusted as shown in Fig. 3. The kernels were thus kept in close con-

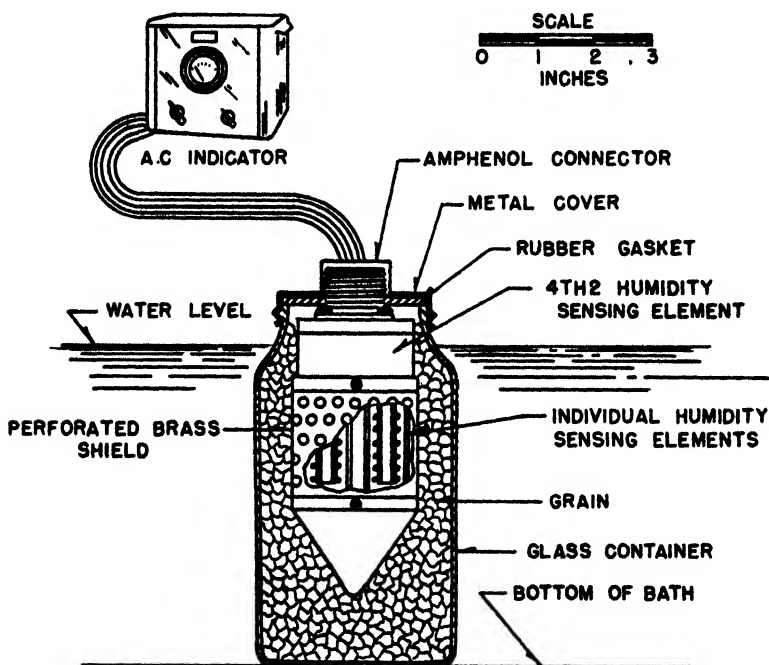


Fig. 3. Diagram showing assembly of apparatus for measuring the equilibrium relative humidity of corn of various moisture contents at constant temperature.

tact with the protective screen which did not interfere with the distribution of the air in contacting the sensitive hygroscopic film of the element, yet kept the grain from making direct contact. Various attempts had previously been made to circulate the air within a container and around the element by using a small turbine-type stirrer dipping into the grain. However, it was impossible to secure consistent humidity readings due to the large volume of air that had to be used in proportion to the small volume of corn, and equilibrium conditions were difficult to obtain. It was found that by keeping the ratio of air to corn low and measuring humidity of the air under static

conditions, more accurate readings could be obtained. Practically no air space was present between the cover and the top of the grain in the jar. Humidity readings were taken at intervals of 15 minutes until no further change in humidity was observed. The temperature and humidity reached equilibrium in about 2 hours.

Moisture Determinations. Brown-Duvel moisture determinations were made by a strictly standardized procedure which involved electrically heating a 100-g. sample of corn in 150 ml. of special moisture tester oil to 190°C. for 20 minutes, allowing to cool to 160°C., and reading the volume of water which had distilled. Single determinations were made by this method, but repeated tests showed that the duplicate determinations did not vary by more than 0.2%.

Duplicate moisture determinations were made by a two-stage vacuum oven procedure (1). The first stage involved drying a 20-g. sample of the whole grain for 0.5 hour in a vacuum oven at 100°C. which reduced the moisture content of all samples below 12%. In the second a 2.0-g. sample of the dried grain, after grinding to pass a sieve with openings 1.0 mm. in diameter, was heated in a vacuum oven at 135°C. for 5 hours.

Results and Discussion

The results are recorded in Table I and are represented graphically in Figs. 4 and 5. The empirical nature of moisture determinations in biological materials is widely recognized. Cook, Hopkins, and Geddes (3) have shown that several methods in common use for grain vary in precision and give different moisture values. They found that, when conducted according to a carefully standardized procedure, the rapid Brown-Duvel method widely used in the grain trade gives reproducible results. In the present study the maximum difference between duplicates was 0.4% by the Brown-Duvel method and 0.2% by the two-stage vacuum-oven method.

The Brown-Duvel method gave lower moisture values than the vacuum-oven method below 17% moisture, whereas the reverse is the case at moistures exceeding this.

Figs. 4 and 5 show that in the region between 12% and 20% moisture, humidity increased rapidly with moisture. At 22% moisture by either method the humidity curve levels off and there is very little change with increased moisture. The ambient humidity tends to approach an asymptotic value at 93% relative. These findings are very similar to results of other workers cited by Oxley (7).

Clean yellow corn of average milling grade at $13.8 \pm 0.2\%$ moisture by the Brown-Duvel method, or $14.7 \pm 0.1\%$ by the two-stage vacuum oven method, is in hygroscopic equilibrium with the ambient air at 75% relative humidity and a dry-bulb temperature of $80^\circ \pm$

TABLE I

RELATION BETWEEN MOISTURE CONTENT AND INTERSTITIAL
RELATIVE HUMIDITY IN WHOLE KERNEL CORN

Sample number	Moisture content		Equilibrium relative humidity	Temp. ²
	Brown-Duvel	Two-stage oven		
	%	%	%	°F
1 ¹	7.0	9.4	40.0	—
2 ¹	7.5	9.9	40.0	—
3	11.6	12.9	59.0	80
4	11.8	12.6	63.5	79
5	12.9	14.1	71.5	80
6	13.2	14.7	71.0	80
7	14.0	15.2	76.5	80
8	14.2	14.6	75.5	80
9	14.6	15.2	79.0	79
10	14.8	15.8	79.0	81
11	15.7	15.8	82.0	78
12	17.3	17.2	85.5	80
13	17.4	17.0	85.0	80
14	17.8	18.2	87.5	81
15	18.0	18.5	86.5	83
16	18.8	18.2	88.0	78
17	18.9	19.4	90.0	79
18	20.0	20.3	90.5	82
19	20.7	19.9	88.5	83
20	22.4	19.5	89.5	83
21	22.5	19.8	91.0	81
22	26.8	22.6	92.0	83

¹ Untempered corn.

² Indicated by resistance.

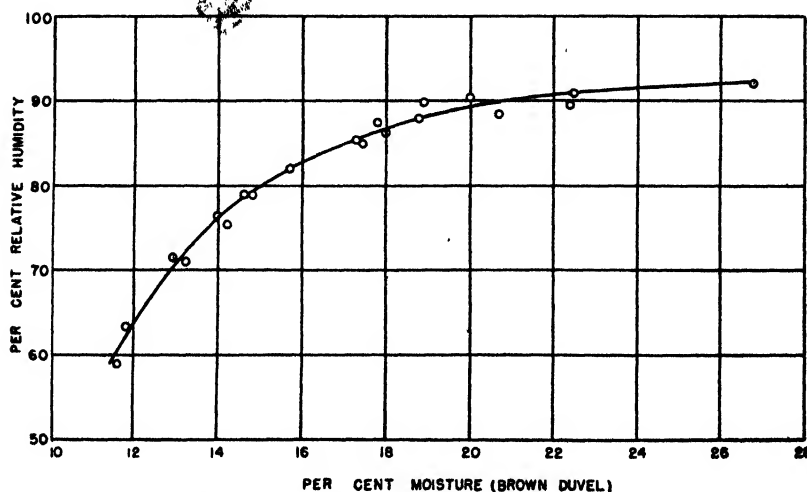


Fig. 4. Relation between relative humidity of interseed atmosphere at 80°F. and the moisture content of shelled whole yellow corn as determined by the Brown-Duvel method. The moisture values are expressed on the wet basis.

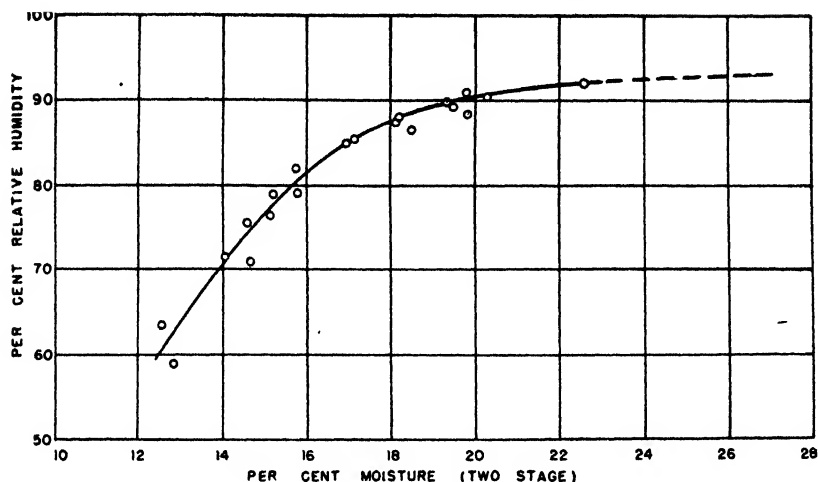


Fig. 5. Relation between relative humidity of interseed atmosphere at 80°F. and the moisture content of shelled whole yellow corn as determined by a two-stage vacuum oven method. The second stage involved heating the ground sample of the previously dried corn for 5 hours at 135°C. The moisture values are expressed on the wet basis.

0.2°F.⁴ As the common molds on grain will not proliferate at humidities below 75%, the moisture content of the grain represented by the above values is the maximum which can be regarded as safe for the storage of corn.

The authors consider that the electric hygrometer employed in this study is the best available method for measuring relative humidity under static conditions or in slowly moving air. Equilibrium readings were more rapidly attained than with other techniques heretofore used and their replicability was very satisfactory.

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⁴ The \pm deviations in moisture and temperature are based on maximum differences between duplicate determinations.

DETERMINATION OF DRY SUBSTANCE IN CONCENTRATED CORN STEEP WATER ¹

BARRETT L. SCALLET and OTTO EBLE ²

ABSTRACT

In a critical study of the benzene distillation method for determination of dry substance in corn steep water it has been shown that the small amounts of acid which distil over with the water do not affect the results. Continuous slow decomposition is believed to take place in the sample during distillation. Water added to dry starch to give a sample containing 60% moisture requires more than 27 hours for complete distillation into a 20 ml. trap. In general, the rate of removal of known quantities of water from dried, remoistened starch is directly proportional to the moisture content. The nearly constant moisture readings obtained with steep water at 20 to 27 hours are thus considered to approach the true moisture values.

The method has been applied to three samples of viscous concentrated steep water obtained at intervals of about one month. The relationships between dry substance values and densities of the diluted solutions were very similar for all three samples.

For a number of years the determination of dry substance in concentrated corn steep water has been a controversial problem. The principal difficulty in determinations is due to the marked instability of the material towards heat. Early determinations of dry substance by drying at atmospheric pressure at 100°C. gave relatively low results because a considerable amount of the dry substance was volatilized. Later determinations at temperatures of 40°–50°C. under vacuum gave higher results, but no definite conclusions about the accuracy of the method could be drawn.

The application of solvent distillation methods to other corn products by Sair and Fetzer (3) indicated the possibility that the true dry substance of steep water can be determined by these methods. The experiments reported here were undertaken to determine whether the benzene distillation method can be used to obtain a value close to the correct dry substance value of steep water.

Materials and Methods

The benzene distillation method of Cleland and Fetzer (2) was used. Samples of steep water were dispersed on dry filtercel, benzene was added, and the flasks were connected to reflux condensers. The standard-taper joints were sealed with Parafilm wax. Leakage was so

¹ Manuscript received December 1, 1947.

² Anheuser-Busch, Inc., St. Louis 18, Missouri.

low by this method that distillation could be continued for 10 days without noticeable loss of benzene. Readings of the water in the traps were made by surrounding the bulbs and graduated portions of the traps with water at 20°C. Readings were taken after 10 minutes, since longer cooling (up to 90 minutes) did not alter the results.

In certain experiments the steep water was neutralized to various pH values with sodium hydroxide solution. For these, a strong sodium hydroxide solution was made up and analyzed for solid content by titration with standard acid. Measured amounts of the caustic solution (about 3–10 ml.) were then added to weighed amounts of steep water (about 150 g.) until the desired pH values were attained (glass electrode). The samples were then analyzed for moisture by benzene distillation.

For the experiments involving neutralization with ammonia, anhydrous ammonia from a cylinder was used. It was passed through two 10-inch drying towers packed with soda-lime and then through a rubber Thomas valve immersed in the weighed steep water. The flow was regulated with a needle valve so that no ammonia escaped into the air. The pH of the steep water was read continuously and the flow of ammonia was stopped when the desired pH was reached. The weight of ammonia added was determined by reweighing the steep water. Moistures were then determined by benzene distillation.

For the experiments in which starch was dried, remoistened, and distilled with benzene, a pearl starch of pH 6.3 was used. Quantities of 36 to 72 g. were weighed out in Erlenmeyer flasks containing a bottom mat of asbestos fiber. The flasks and contents were then dried to constant weight in the Weber oven at 100°C. and 5 mm. of mercury pressure. Total drying time was 48 hours (three 16-hour periods; weight constant after 32 hours). About 19 ml. of water were added to each flask, in such a way that the starch was exposed to the atmosphere for only about 5 seconds; the weight was redetermined, and the water then distilled with benzene.

Traps were calibrated only at the highest graduation. However, sample sizes were selected so that the final readings were very close to the top graduation.

For the series of steep water dilutions, a sample for which the dry substance value had been determined by benzene distillation was diluted progressively with water. A weighed sample of steep water was diluted quantitatively by adding water and reweighing. It was then cooled to 60°F. and the Be. reading determined. The sample was returned to the original beaker, reweighed, and diluted further in the same manner. The range covered was 3°–22° Be.

Results and Discussion

Effect of pH of Sample on Moisture Recovery. In applying the standardized benzene distillation method of Cleland and Fetzner (1), preliminary results with 5 ml. traps indicated that there was a period of near constancy from 3 to 9 hours after the start of distillation. However, the water recovered in the distillate was definitely acid and required several ml. of 0.1 *N* sodium hydroxide to neutralize it to a phenolphthalein end point. This indicated that some of the acids of the steep water were volatile with benzene-water.

Although it was suspected that the influence of this amount of acid on the volume of water recovered was negligible, this point was tested by neutralizing a sample of steep water with sodium hydroxide solution to various pH values before distillation. Since water was added with the sodium hydroxide and water was formed in the neutral-

TABLE I
EFFECT OF pH OF SAMPLE ON RECOVERY OF MOISTURE
FROM STEEP WATER

	pH					
	3.6	5	6	7	8	9
Dry substance, % (duplicates)	— 46.2	48.0 49.4	47.8 47.5	49.3 48.4	47.8 48.3	48.6 48.4
Ml. 0.1 <i>N</i> sodium hydroxide to neutralize distillate	0.90	0.10	0.05	—	—	—
Ml. 0.1 <i>N</i> sulfuric acid to neutralize distillate	—	—	—	0.10	0.12	0.14

ization of acids with the base, a rather involved calculation was necessary to bring all the results to the original steep water basis (Table I).

The results showed that the distillate would be neutral at a sample pH of about 6.3. At higher pH values ammonia was evolved in sufficient quantities to make the distillates alkaline. The dry substance results were too erratic to permit definite conclusions about the pH effect. In this experiment 5 ml. traps were used and the sample weight was about 6 g. The uncertainty in trap readings was about ± 0.02 ml. and an error of 0.01 ml. corresponded to a dry substance error of 0.3%. To increase the precision of the method, 20 and 35 ml. traps were used. The uncertainty of readings was also ± 0.02 ml. for these traps, but much larger samples were used. With a 20 ml. trap and a sample weight of about 40 g., the ultimate precision of the

method should be about 0.1%. The 35 ml. traps should give 0.06% precision.

In actual operation on steep water the precisions obtained were about 1% for 5 ml. traps, 0.4% for 20 ml. traps, and 0.6% for 35 ml. traps.

The moisture contents of neutralized and unneutralized samples of steep water were next determined side by side in 5, 20, and 35 ml. traps. To eliminate difficult calculations and simplify experimental procedure, anhydrous ammonia was used to neutralize half of the

TABLE II
EFFECTS OF NEUTRALIZATION WITH AMMONIA AND OF TRAP SIZE
ON RECOVERY OF WATER

Distillation time	Moisture content					
	Trap size, ml.					
	5		20		35	
	pH					
	3.6	6.4	3.6	6.4	3.6	6.4
<i>Hour</i>	%	%	%	%	%	%
1.5	70.77	70.61	67.49	70.71	—	—
6	71.72	71.62	71.31	71.37	71.07	70.84
8	71.72	71.77	71.36	71.56	71.16	70.88
21	72.20	72.20	71.80	71.94	71.63	71.58
28	72.36	72.20	71.94	72.08	71.79	71.64
48	—	—	—	72.12	72.01	71.77
68	72.52	72.35	—	—	—	—
93	72.67	72.64	72.29	72.40	72.31	72.01
Ml. 0.1 <i>N</i> sodium hydroxide to neutralize distillate	1.17	0.00	2.00	0.00	4.88	—
Ml. 0.1 <i>N</i> sulfuric acid to neutralize distillate	—	—	—	—	—	15.1

sample of heavy steep water to pH 6.4. The results (Table II) show that the pH of the steep water has no recognizable effect on the determination of dry substance. The variations between columns are of the same order as the experimental error. The quantity of acid distilled from the samples at pH 3.6 was not enough to affect the trap readings. The moisture value attained with the 35 ml. traps in 48 hours was attained in 21 hours with the 20 ml. traps and in 8 hours with the 5 ml. traps. However, no period of constant readings was found with any of the samples.

Rate and Completeness of Moisture Removal. Because the periods between readings were long in the previous experiment, any possible plateau in the water-removal curve would probably have been missed. Since there were indications that a plateau might exist at about 20

TABLE III

RATE OF MOISTURE REMOVAL DURING BENZENE DISTILLATION OF FOUR SAMPLES OF STEEP WATER OF DIFFERENT MOISTURE CONTENTS¹

Distillation time	Moisture collected			
	Sample A ¹	Sample B ¹	Sample C ¹	Sample D ¹
<i>Hours</i>	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>
1.5	17.03	18.16	15.88	15.00
1.75	17.32	18.39	17.09	16.22
2	17.36	18.46	17.47	16.54
2.6	17.43	18.52	17.73	16.87
4.2	17.51	18.64	17.84	16.99
5.2	17.54	18.70	17.93	17.02
13	17.61	18.78	17.98	17.16
14	17.62	18.79	17.99	17.17
15.5	17.64	18.81	18.02	17.20
16	17.65	18.83	18.04	17.20
17	17.66	18.84	18.04	17.20
18	17.66	18.84	18.04	17.23
19	17.66	18.84	18.06	17.22
20.5	17.69	18.87	18.06	17.24
21	17.68	18.86	18.06	17.24
22	17.69	18.86	18.07	17.25
23	17.68	18.87	18.06	17.25
24	17.69	18.88	18.07	17.26
25	17.69	18.88	18.07	17.27
26	17.69	18.88	18.08	17.26
27	17.69	18.88	18.08	17.26
40	17.73	18.93	18.13	17.33
41	17.73	18.92	18.12	17.33
42	17.73	18.92	18.12	17.33
66.5	17.75	18.95	18.15	17.36
90.5	17.77	18.96	18.18	17.38
115	17.79	18.97	18.21	17.39
164	17.82	19.00	18.23	17.43

¹ Densities of the four steep waters after diluting with equal weights of water and weights of the samples taken for distillation were:

Sample No.	°Be.	Sample weight g.
A	12.15	20.816
B	13.05	23.726
C	14.50	23.486
D	16.10	23.309

hours with the 20 ml. traps, another experiment was performed (Table III) in which trap readings were taken at hourly intervals from 13 to 27 hours after the start of distillation. The samples were taken at successive stages during the concentration of a batch of steep water.

The results showed only a gradual removal of water, with no extended period of constancy of readings that could be attributed to

completion of removal of water before decomposition set in. It seemed quite probable that a slow decomposition was occurring throughout the distillation, and that the nearly constant readings from 20 to 27 hours gave values close to the true moisture contents of the samples.

To test this point another experiment was performed to find the length of time necessary to remove a known amount of water completely by benzene distillation. An organic material stable to heat and capable of holding water at least as tenaciously as steep water was needed, and corn starch was judged to meet these requirements. Samples of pearl starch of pH 6.3 were dried to constant weight at 100°C. under high vacuum. They were then remoistened with

TABLE IV

RATE OF REMOVAL OF WATER FROM STARCH OF KNOWN MOISTURE CONTENTS

Distillation time	Percentage recovery of water from starch of various moisture contents ¹			
	30%	40%	50%	60%
<i>Hours</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>
4	—	—	—	97.77
5.5	—	—	—	99.04
7.5	—	—	—	99.41
10	92.15	—	98.13	—
16	94.79	—	99.20	—
21	95.85	98.54	99.09	—
27	—	—	—	99.67
41	98.07	99.81	100.05	—
69	99.13	100.23	100.21	—
113	99.60	100.50	100.26	—
210	99.92	100.55	100.31	—

¹ Weighed amounts of water were added to pearl starch (dried to constant weight at 100°C. under vacuum) to give the moisture values indicated. Sample weights for the distillation were so selected that 19 g. of water were present in each case.

weighed amounts of water and subjected to benzene distillation. Sample weights were selected so that the addition of 19 g. of water to each would yield wet starches of 30, 40, 50, and 60% moisture contents.

The results (Table IV) show that moisture removal is dependent upon the amount of dry substance present tending to retain it. The greater the amount of starch present, the slower is the removal of water. All of the added water was removed from the starch containing 60, 50, 40, and 30% moisture in the following respective times: 27, 41, 41–69, and 210 hours. Water removed in excess of that added may have been due to slight decomposition of the starch.

Application of these results to the determination of moisture in steep water shows that the observed behavior of steep water during

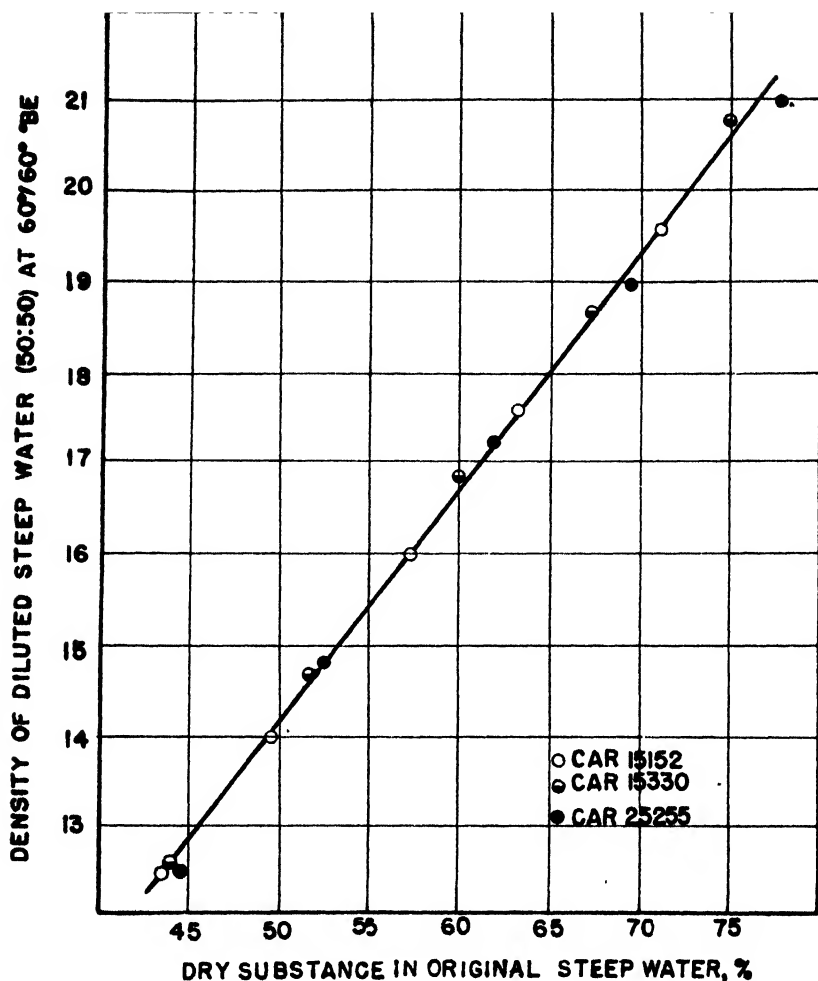


Fig. 1. Curve relating dry substance and Baumé of 50:50 (by weight) dilution of heavy steep water samples.

distillation can be explained by the same sort of slow decomposition. It is thus highly probable that the period of near constancy of moisture readings from 20 to 27 hours (with 20 ml. traps) for steep water of about 70% moisture content gives a value very near to the true moisture value of the steep water.

Application of the Method. The method of determination of dry substance by means of hydrometer readings is generally rapid, reproducible, and relatively accurate. The readings must of course be related to the dry substance by some absolute method such as benzene distillation.

In the case of many concentrated steep waters the hydrometer method is difficult to apply because of the viscous, semicrystalline state of the material.

However, the hydrometer method can be applied readily to diluted samples of this type of steep water. A dilution of 50:50 by weight was chosen for the following reasons:

(a) The physical nature of the 50:50 dilution is such that an accurate Be. reading can be obtained easily and quickly.

(b) A 50:50 dilution rather than a greater or smaller dilution reduces the effect of weighing errors to a minimum.

(c) A dilution by weight rather than volume is more accurate for the semisolid material.

(d) The per cent dry substance value for the dilution is exactly half of the value for the original solution, which simplifies calculations.

Dry substances (determined by benzene distillation) of several steep water samples are plotted against the densities of 50:50 dilutions in Fig. 1. The points fall on a reasonably straight line in this Be. range. This curve applies only to incubated steep water (dextrose converted to lactic acid with *Lactobacillus*) which has a Be.-dry substance relationship considerably different from that of the nonincubated type. Experience with samples from other companies indicates that steep water varies from company to company and from time to time for a given company. The curve shown was obtained with samples from tank cars which represented a number of batches. The three samples were obtained at intervals of about a month during the fall and show that the steep water is reasonably constant during a season. The relationship should be verified perhaps as often as four times a year.

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SOLUBILITY STUDIES ON THE NITROGENOUS CONSTITUENTS OF SPENT BREWERS' GRAINS¹

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ABSTRACT

The proteins in dried spent brewers' grains are poorly soluble in the common protein solvents. Less than 10% of the grain nitrogen is extractable with water, 10% aqueous sodium chloride, 0.075 *N* hydrochloric acid, or 0.01 *N* sodium hydroxide. After ball mill grinding of the grains, prolonging the extraction time to several hours, and using 0.5 *N* sodium hydroxide as the solvent, up to 90% of the grain nitrogen can be peptized. However, these conditions also favor the dispersion of the nonnitrogenous constituents of the grains. The net effect is only an insignificant increase of the nitrogen content in the isolated product over that of the starting material. About 75% of the dispersed nitrogen is precipitable at either pH 3.75 or pH 4.55, and about 85% in the presence of 30% of either sodium sulfate or magnesium sulfate ($\cdot 7 \text{ H}_2\text{O}$) at pH 4.1. The isolated material contains, on an ash and moisture free basis, 69.3% carbohydrates, 4.75% nitrogen, no cystine, 0.65% methionine, and 8.55% glutamic acid.

In the terminology of the brewing industry, spent brewers' grains comprise the residue of the barley malt after germination, removal of the sprouts, mashing, and drawing off of the mash liquor (the wort) from the solids (the spent brewers' grains). The wet spent grains are customarily first pressed, which reduces the moisture from 80% to 65–70%, then dried either by passing hot air through them, or by exposing them to rotating peripheral steam coils or chests (4). The moisture is thereby reduced to below 10%. The dried grains are sold as animal feeds. During 1940–41, 106,000 tons of dried spent brewers' grains reached the market; the amount increased to 230,700 tons in 1943–44, but dropped slightly to 211,900 tons during 1945–46 (1). Since the average crude protein content of the grains amounts to slightly over 25%, it seemed of interest to investigate the feasibility of recovering the proteins for use in industrial processes.

Experimental and Results

The dried spent brewers' grains used in this investigation were taken from a typical commercial lot. They contained 5.3% moisture, 2.6% ash, 25.5% protein (4.13% $\text{N}^2 \times 6.25$), and 6.25% ether extractables.²

¹ Manuscript received December 6, 1948.

From the research laboratories of the Amino Products Division of International Minerals and Chemical Corporation, Rossford, Ohio.

² All analytical results throughout this report are calculated on an ash and moisture free basis.

TABLE I
PEPTIZATION OF SPENT BREWERS' GRAIN NITROGEN DURING
ONE-HOUR EXTRACTION PERIODS

Solvent used	pH after shaking	Meal nitrogen extracted
		%
90% (conc.) Formic acid	0.0	34.1
Glacial acetic acid	1.0	17.7
0.075 <i>N</i> Hydrochloric acid	1.7	4.3
10% Sodium chloride	5.4	3.1
Water	5.5	2.9
0.01 <i>N</i> Sodium hydroxide	9.2	8.1
0.1 <i>N</i> Sodium hydroxide	11.7	35.2
0.5 <i>N</i> Sodium hydroxide	11.6	52.0
1.0 <i>N</i> Sodium hydroxide	11.4	51.9

Protein extractions were performed in 250 ml. centrifuge bottles. One hundred ml. of solvent were added to 3 g. of grains and shaken for the desired length of time on a mechanical shaker. The bottles were centrifuged, the supernatant liquid filtered, and aliquots were analyzed for nitrogen by the micro Kjeldahl method with a mercuric oxide catalyst.

Table I records the pH of the extracts, and the percentage of nitrogen peptized during a 1-hour shaking period. Water, 10% aqueous sodium chloride, 0.075 *N* hydrochloric acid, or 0.01 *N* sodium hydroxide peptize less than 10% of the grain nitrogen, while glacial acetic acid, 90% formic acid, or 0.1 *N* sodium hydroxide disperse up to 35% of the nitrogen. Increasing the strength of the alkali to 0.5 *N* peptizes a maximum of 52% of grain nitrogen.

Water does not extract materially more nitrogen by lengthening the shaking time from 1 to 4 hours (Table II), but 0.5 *N* sodium

TABLE II
EFFECT OF LENGTH OF SHAKING TIME ON NITROGEN PEPTIZATION OF SPENT
BREWERS' GRAINS WITH WATER OR 0.5 *N* SODIUM HYDROXIDE

Solvent used	Time of shaking	Meal nitrogen extracted
		%
Water	1	2.9
Water	2	3.2
Water	3	3.2
Water	4	3.3
0.5 <i>N</i> Sodium hydroxide	1	52.0
0.5 <i>N</i> Sodium hydroxide	2	57.9
0.5 <i>N</i> Sodium hydroxide	3	62.2
0.5 <i>N</i> Sodium hydroxide	4	68.0
0.5 <i>N</i> Sodium hydroxide	6	74.4
0.5 <i>N</i> Sodium hydroxide	16	90.6

hydroxide as the solvent raises the nitrogen dispersion from 52% at 1 hour to 74.4% after 6 hours, and to 90.6% after 16 hours' contact time.

Spent brewers' grains, as they reach the market, are of a coarse texture. Ball-mill grinding of the grains for 8 hours at 70 r.p.m. permits a more efficient nitrogen peptization. With 0.5 *N* sodium hydroxide as the solvent 74.4% of the nitrogen is dispersed in 1 hour, and 93.0% after 5 hours shaking (Table III).

The percentage of nitrogen which is removed by high speed centrifugation (about 3,500 r.p.m.), followed by filtration of the supernatant solutions of 0.5 *N* sodium hydroxide extracts of ball-mill-ground grains, after pH adjustment with hydrochloric acid, is shown in Table IV. Two regions of minimum solubility are evident, one at or near pH 3.75 and a second at or near pH 4.55. A maximum of about 75% of the dispersed nitrogen can be recovered at either pH after

TABLE III
EFFECT OF PARTICLE SIZE OF SPENT BREWERS' GRAINS ON NITROGEN
PEPTIZATION WITH 0.5 *N* SODIUM HYDROXIDE
FOR VARYING EXTRACTION PERIODS

Extraction time	Nitrogen peptized from meal	
	Original sample	Ball-mill ground
<i>Hours</i>	%	%
1	52.0	74.1
3	62.2	83.5
4	68.0	87.3
5	—	93.0
6	74.4	—

an overnight flocculation of the precipitate, followed by centrifugation and filtration of the supernatant liquid. The two solubility minima probably indicate the existence of at least two protein fractions, each with a different isoelectric point. The closeness of the two isoelectric points suggested the use of salts at a pH value half way between the pH minima in order to increase protein recovery. Sodium chloride at pH 4.1, in 30% concentration, did not raise nitrogen recovery; however, either sodium sulfate or magnesium sulfate ($\cdot 7 \text{ H}_2\text{O}$) in 30% concentrations precipitates an additional 10% of the extracted nitrogen, i.e., a total of 85%.

The precipitate which forms after pH adjustment of the extracts is slimy, and while it packs reasonably well in 50 ml. cups at high centrifugal speeds (about 3,300 r.p.m.) it does not pack in 500 ml. cups at lower speeds (2,000–2,300 r.p.m.). Filtration instead of centrifugation requires frequent changes of filter paper because the slimy film which

settles on the filter paper quickly prevents further passage of liquid. Consequently, when an attempt was made to isolate the precipitated product from a large-scale extraction, yields were much poorer than one would surmise from the theoretical extraction and precipitation data shown in Tables III and IV. An analysis was made of the precipitate obtained from 0.5 *N* sodium hydroxide extracts of ball-mill-ground spent brewers' grains after the addition at pH 4.1 of 30% sodium sulfate. Only 10.2 g. of dried material (calculated on an ash-free and

TABLE IV
EFFECT OF pH ON NITROGEN PRECIPITATED FROM 0.5 *N* SODIUM HYDROXIDE
EXTRACTS OF BALL-MILL GROUND SPENT BREWERS' GRAINS

Substance added	pH of solution after 16 hours standing	Extracted nitrogen precipitated %
Hydrochloric acid	3.5	63.4
Hydrochloric acid	3.7	74.9
Hydrochloric acid	3.75	75.8
Hydrochloric acid	3.85	70.8
Hydrochloric acid	4.0	68.8
Hydrochloric acid	4.2	67.6
Hydrochloric acid	4.35	69.6
Hydrochloric acid	4.55	74.7
Hydrochloric acid	4.6	71.4
Hydrochloric acid	4.8	50.9
AFTER SALT ADDITION ¹		
Sodium chloride	4.1	75.1
Sodium sulfate	4.1	85.5
Magnesium sulfate ($\cdot 7 \text{ H}_2\text{O}$)	4.1	85.6

¹ Each salt was added in sufficient quantity to yield a final concentration of 30%.

moisture-free basis) was obtained from 100 g. of spent grains, while theoretically 60 g. should have been the yield $\left(\frac{(4.13)(0.93)(0.855)(100)}{100} \right)$

After the addition of salts these unfavorable characteristics of the extracts are further accentuated.

The precipitate was dried under infrared light. A slightly hygroscopic, dark tan powder was obtained. It contained 36.8% ash and 16.4% moisture, the latter determined by further drying of a sample in an Abderhalden vacuum pistol over phosphorus pentoxide at the temperature of boiling toluene. The product contained 69.3% carbohydrate (2), 4.75% nitrogen, no cystine (6), 0.65% methionine (3), and 8.55% glutamic acid (5) ².

² The glutamic acid analyses by the decarboxylase method were performed by Murry Seidman of this laboratory, for which grateful acknowledgment is made.

Discussion

The poor solubility of the nitrogenous constituents of spent brewers' grains could be a characteristic property of the original constituents of the grains, which have been modified by malting and mashing. More likely, however, the lack of solubility is a result of the customary drying methods of either passing hot air through the wet grains, or of exposing them to rotating steam coils. These are ideal conditions for protein denaturation. It is surmised that if drying conditions could be devised which would avoid prolonged exposure of the wet grains to elevated temperatures, the proteins of the spent brewers' grains would probably lend themselves more readily to industrially applicable extraction and purification procedures, similar to those now used on oil-seed meals. Whether drying of spent brewers' grains under such conditions is economically feasible is, however, doubtful.

The drastic conditions of ball-mill grinding, relatively high alkalinity of the solvent, and prolonged contact periods of the grains with the solvent, necessary to peptize the nitrogen of commercial spent brewers' grains, also favor the dispersion of the other plant cell constituents. The net effect of the attempted purification is therefore an insignificant raising of the nitrogen content of the isolated "protein" from that of the starting material, namely 4.75% against 4.13% nitrogen, respectively. The complete absence of cystine in the isolated product does not necessarily indicate the absence of cystine in intact spent brewers' grains. It appears more likely that cystine with its known sensitivity to alkali was destroyed during the long exposure to 0.5 *N* sodium hydroxide.

Acknowledgment

This investigation was suggested by Dr. M. J. Blish, Supervisor of Protein Research of International Minerals and Chemical Corporation. The authors wish to express their appreciation to Dr. Blish for his interest throughout this investigation.

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COMMUNICATION TO THE EDITOR¹

A Method for Grinding Bread Crumb

SIR:

A method for preparing bread crumb for analysis may prove of interest since to our knowledge a similar procedure has not appeared in the literature.

The high moisture content and soft texture of bread crumb make it difficult to grind or otherwise prepare for analysis. Bread-crumb can, however, be quickly cut or broken into pieces approximately 2 cm. square, or smaller, and these can be rapidly frozen at -30°C . in a deep freeze unit. This procedure retards biochemical and biological reactions in the bread and the sample can then be stored until convenient to proceed to the next step.

The frozen crumb is ground on a Wiley mill by mixing it with dry ice (solid carbon dioxide). The mill is first chilled by grinding dry ice and then the mixture of dry ice and bread crumb is put through the mill. By this means bread crumb can be finely ground without appreciable change in moisture content. The method is a convenient, rapid, and satisfactory way of obtaining material for the study of bread-staling rates by the water-absorption and viscosity methods. The residual dry ice quickly evaporates, at room temperature, from the ground sample.

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BOOK REVIEWS

Les Succédanés en Panification (Substitutes in Breadmaking). By H. Nuret and R. Calvel with the collaboration of M. Dubois and the staff of the Laboratoire d'Études et de Contrôle de la Qualité des Blés Français. 115 pp. Published by the Association of Former Students of the French School of Milling, 16 rue Nicolas Fortin, Paris, France. 1948.

This brochure presents interesting and well-organized data concerning the many problems encountered by French bakers during and since the war as a result of the shortage of wheat and the necessity of using long-extraction flour together with numerous substitutes. Except for the periods of the last world wars and since that time, the bread made in France had an enviable reputation and constituted a large part of the diet of the people. Unlike most of the bread in this country, French bread is made from the basic ingredients of flour, water, yeast, and salt, without the addition of milk solids, malt, sugar, shortening, and yeast foods. In the spring of 1948, the shortage of wheat obliged the Government and the bakers to use substitutes for wheat flour in greater quantities than at any previous time. This state of affairs induced the Government to order a study of the many technical problems caused by such additions.

¹ Received November 26, 1948.

The booklet gives a good description of the three principal breadmaking methods used in France. This description is followed by a chapter outlining the regulations and the composition of flours used in France from November, 1940, to November, 1947. The influence of long-extraction flours (up to 98%) on breadmaking characteristics and the general adjustments needed in procedure are briefly discussed. The main part of the brochure describes results obtained using the following substitutes for wheat flour:

1. Cereals: corn, barley, rye, oats, buckwheat
2. Legumes: peas, soybeans, peanuts, beans
3. Potatoes: mashed potatoes, raw crushed potatoes, potato flour
4. Corn starch, potato starch

Details concerning the effect of each of the above additions, in selected amounts from 10 to 50%, on the procedures and the finished bread as compared with the standard are given in a clear, concise manner. The alveograph was used to evaluate some of the mechanical properties of the doughs and, in addition, data are listed on the protein, ash, fat, and diastatic activity values of the various admixtures. The material is presented in the language of the baker with major emphasis on the adjustments necessary to produce the best bread possible from ill-suited but economically necessary substitutes for wheat flour. The technical data are sound and the booklet can be read with profit by anyone interested in baking.

The baking industry of the United States was faced with many trials during the war, but difficulties with 80% extraction flour and shortages of milk solids, fats, and sugars fade into insignificance when compared with the unsurmountable problems of the bakers of France in trying to produce a palatable loaf of bread. Naturally, with the materials they were forced to use, their bread has been poor. It is hoped that the day is near when the good bread of France will return to sustain her people.

BETTY SULLIVAN

Russell Miller Milling Company
Minneapolis, Minnesota

Test Baking. By J. D. Mounfield. 32 pp. The Northern Publishing Company, Ltd., Liverpool 1, England. 1948. Price, 3 shillings.

The author states in the preface that this booklet on test baking is intended mainly as an aid to those engaged in controlling flour quality in mill or bakery. For this purpose it should prove helpful. There are three chapters, namely, "Planning the Test," "Doing the Test," and "Assessing the Result." There is also an appendix containing brief instructions for the statistical treatment of data.

The author has emphasized the importance of having a definite object in mind when planning a baking test and when evaluating the results. He also calls attention to the fact that most of the manipulations in the baking process and the characteristics of the bread produced are matters upon which opinion can, and frequently does, differ greatly. It is lamented that a means has not been found to measure each separate feature of a loaf mechanically and record the result numerically.

The cereal technologist who has followed the development of the baking test as published in the various issues of *CEREAL CHEMISTRY* would not have much incentive to read *Test Baking*, except perhaps to become better acquainted with a British point of view. The contrast between the problems faced by test bakers in England compared with North America is stressed, and an explanation is given why a standard baking test has not been developed in England.

It is unfortunate that the only references to the official A.A.C.C. baking test are to an article in *CEREAL CHEMISTRY* (1934) and to *Cereal Laboratory Methods*. Apparently the author is not aware that this book is now in its fifth edition and that the baking test has been modified drastically with time.

The booklet is published with paper covers, the printing is on a good grade of paper, and the print is easy to read. Several illustrations of bread are included.

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EFFECT OF HEAT TREATMENT OF SEPARATED MILK ON THE PHYSICAL AND BAKING PROPERTIES OF DOUGHS ENRICHED WITH DRY MILK SOLIDS¹

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ABSTRACT

The three major components of nonfat dry milk solids, casein, lactose, and the milk serum proteins, were heated in solution for 30 minutes at 70°C., 75°C., and 80°C., and incorporated into doughs in amounts equivalent to the addition of 6% of nonfat milk solids. The unheated fractions all depressed loaf volume, with lactose having the greatest effect. In the absence of potassium bromate, heat treatment improved the baking quality of the milk serum proteins considerably but had no effect on the baking behavior of casein or lactose. Inclusion of bromate in the baking formula increased the loaf volume when unheated milk serum proteins were added to doughs; bromate also somewhat improved the baking results with lactose.

Farinograms and extensograms for salt-water doughs showed that the inclusion of either nonfat milk solids prepared from raw separated milk or unheated serum proteins caused marked softening upon prolonged mixing, greatly increased extensibility, and a lowered resistance to extension. Heat treatment overcame these effects.

Improvement in the baking quality of separated milk is a function of both the time and temperature of heating. The minimal conditions for maximum improvement were found to be 73°C. for 30 minutes, about 85°C. for 7 minutes, and 92°C. for the flash method (about one minute) of heating.

Several studies have been made in these laboratories of the factors that influence the baking quality of nonfat milk solids since the pioneer work of Greenbank *et al.* (4) and of Grewe and Holm (5) showed that heat treatment of separated milk prior to drying improves the baking quality of the resulting nonfat milk solids. Skovholt and Bailey (11) demonstrated that heat treatment of separated milk reduces the tendency of doughs to slacken upon extended mixing. The farinograph

¹ Manuscript received December 28, 1948. Presented at the Annual Meeting, May, 1948.

Paper No. 2312, Scientific Journal Series, Minnesota Agricultural Experiment Station. Some of the data in this paper were taken from a thesis presented to the Graduate School of the University of Minnesota by R. A. Larsen in partial fulfillment of the requirements of Doctor of Philosophy, June, 1947.

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studies of Stamberg and Bailey (13) indicated that the dough-softening factor is concentrated in the whey portion of milk, and is heat-labile. Employing the Heyrovsky micropolarograph, they found that fresh separated milk exhibited a wave characteristic of sulfhydryl compounds, whereas boiled milk failed to show this wave. Sulfhydryl compounds are known to cause dough softening and as the sulfur content of the serum proteins is much higher than that of casein (Baernstein, 1), these observations, although unconfirmed by baking tests on the milks or milk fractions, suggest that the heat improvement of separated milk for breadmaking primarily involves the serum proteins and probably is associated with a decrease in the amount or activity of sulfhydryl groups in these proteins.

It appeared logical to subject separated milk, and fractions prepared from it, to a series of controlled heat treatments and to study the baking effects and the biochemical properties of the various fractions. Should it be established that the improvement is associated with certain biochemical changes in a particular fraction, a simple test for production control purposes might evolve. Such a development would be of considerable practical significance since baking tests are empirical and time consuming, and the vast majority of producers of nonfat milk solids do not have the facilities for carrying them out. During the progress of this research, Harland *et al.* (6) have reported baking experiments undertaken with a similar objective.

The present paper deals with the influence of heat treatment of casein, serum protein, and lactose on their effects on loaf volume and on the physical properties of doughs. The relation of time and temperature of heating skim milk to the baking behavior of nonfat milk solids prepared from it was also investigated.

Materials and Methods

Fresh separated milk produced from bulked milk of the University dairy herd was used throughout this study with the exception that the series of flash-heated samples was supplied by a commercial organization.

Milk Fractions. Since heat treatment of milk results in the partial precipitation of the milk serum proteins with the casein upon subsequent fractionation (7, 10), it was necessary to make the protein separations prior to heat treatment to secure pure fractions.

Casein was prepared from the separated milk by isoelectric precipitation according to the method of Van Slyke and Baker (17) and purified by washing, redispersion in 5% ammonium hydroxide solution to pH 6.6, reprecipitation, and washing. For the purpose of heat treatment, the purified casein was reconstituted as a calcium phos-

phocaseinate solution. This was accomplished by triturating 2.5 g. casein, 51 mg. hydrated trisodium citrate, 100 mg. hydrated tripotassium citrate, and 50 mg. calcium hydroxide in a mortar. Seventy-five milliliters of clear saturated calcium hydroxide solution were slowly added followed by the dropwise addition, with thorough mixing, of 13.25 ml. of 1.0% orthophosphoric acid solution containing 0.3% calcium hydroxide and 0.635% monocalcium citrate. The solution was then made up to 100 ml., yielding one of the same caseinate concentration and pH as milk.

The milk serum protein fraction for the baking tests was prepared from fresh raw separated milk at 25°C. (or 35°–40°C. in some cases) by adding 100 ml. of 10% acetic acid solution per liter, holding for 15 minutes, and adding 100 ml. of 1.0 *N* sodium acetate solution per liter. After 10 minutes the precipitated casein was removed by filtering or centrifuging. The serum was dialyzed free of water-soluble constituents and buffered with phosphate buffer to a pH of 6.6 and ionic strength of 0.1 in the final solution (adjusted to a protein concentration of about 0.7 g. per 100 ml.).

The milk serum protein for the physical dough tests was prepared by precipitation of the casein from fresh skim milk by adjusting the pH to 4.6 with 4% hydrochloric acid solution, followed by dialysis⁶ of the serum against distilled water. Aliquots of this solution were adjusted to a concentration of 0.7 g. of protein per 100 ml. with distilled water and heat-treated as described in the next section.

Heat Treatment. Heat treatments of skim milk and solutions of calcium phosphocaseinate, serum protein, and lactose⁶ were carried out at 70°, 75°, and 80°C. in a thermostatically controlled water bath. The preparations were held in the bath for 30 minutes after they had attained the selected temperature and then rapidly cooled by running water.

Concentration of the preparations was effected by pervaporation in Visking sausage casings. Some of them were dried from the frozen state under vacuum in a chamber containing alumina for a desiccant.

Nonfat Milk Solids. Three lots of spray-dried nonfat dry milk solids were prepared from bulked separated milk which had been preheated by different methods. For the first series, sublots from one source were heated in a steam-jacketed vat for 30 minutes at temperatures ranging from 63°C. to 85°C. The lots of the second series were heated in a similar manner for 7 minutes at temperatures varying from 68°C. to 82°C. The third series was flash-heated in Grey-Jensen

⁶ This dialysis was not exhaustive. The preparations contained about 75% protein on the dry basis. The milk serum protein for the physical dough tests was prepared some months prior to that used for baking. The precipitation of the casein with acetate buffer is more convenient than with hydrochloric acid, but the two methods yield serum protein of identical characteristics.

⁶ A solution containing 6 g. of U.S.P. lactose per 40 ml. was used throughout these studies.

equipment at a series of temperatures from 77°C. to 110°C. All samples were spray dried in conventional type driers, packed under nitrogen in cans, and stored at -5°C. until used in baking studies.

Baking Tests. Several studies have shown that the inclusion of nonfat milk solids in the baking formula increases the dough-mixing requirements, the fermentation tolerance, and also the bromate requirement and bromate tolerance; moreover, the effect of nonfat milk solids on bread quality is influenced by the characteristics of the flour.

A short study was undertaken with two commercial samples of nonfat milk solids, one of high and the other of low quality, to develop a suitable formula and baking procedure for evaluating the samples of nonfat milk solids and milk fractions. The variables which were investigated included flour strength, baking method (straight dough vs. sponge and dough), percentage of nonfat milk solids, bromate level, and severity of punching. The best differentiation between the nonfat milk solids of good and poor quality was obtained with a very strong bakers' patent hard red spring wheat flour (14.5% protein on a 14.0% moisture basis), the addition of 12% of nonfat milk solids and 2.0 mg. of potassium bromate per 100 g. of flour to the ingredients of the A.A.C.C. basic formula, a mixing time of 3 minutes (Hobart Swanson mixer), a 2-hour fermentation, and a single passage of the dough through a National dough sheeter (set at 9/16 inch) at the time of molding. The baking tests in the present study, however, were conducted mostly with 6.0% of nonfat milk solids or their equivalent to reduce the labor involved in preparing the fractions. In most cases, also, a mixing time of 2.5 minutes was used. Each sample was baked at least in duplicate with 0, 1, and 2 mg. of potassium bromate respectively per 100 g. of flour and the standard errors of the loaf volumes were calculated for each series to aid in interpreting the data. By this baking procedure, commercial nonfat milk solids of poor quality gave loaf volumes averaging about 10% lower than those obtained when nonfat milk solids of good baking properties were used.

Physical Dough Tests. Farinograms and extensograms were made at 30°C. with salted doughs containing 12% of nonfat milk solids or equivalent amounts of the various fractions (4.3% of casein, 0.9% of serum proteins, or 6.0% of lactose). Two per cent of sodium chloride was used and the proper amount of water was added to the heat-treated products to give a maximum dough consistency of 500 units. The absorption determined for each heat-processed material was employed in the farinogram test on the corresponding untreated samples. The total weight of dough was adjusted in all cases to 480 g. as recommended by Stamberg and Merritt (14). For the extensograph tests, doughs were mixed in the farinograph as already described, rounded

and molded in the extensograph, and then placed in the dough trays where they were held at 30°C. for 45 minutes and an extensogram made. Two additional extensograms were made on the same dough at successive intervals of 45 minutes as described by Merritt and Bailey (8).

Results

Effect of Milk Fractions and Their Heat Treatment on Loaf Volume.

The effects of the three milk fractions, with and without heat treatment, on loaf volume are shown in Table I. Casein, milk serum pro-

TABLE I

EFFECT OF HEAT TREATMENT OF CASEIN, MILK SERUM PROTEIN, AND LACTOSE ON LOAF VOLUME OF BREAD BAKED AT VARIOUS BROMATE LEVELS

(Amount of each fraction per loaf equals that present in 6 g. of nonfat milk solids)

Milk constituent	Potassium bromate ¹	No. of replicates	Mean loaf volume ² for samples heated for 30 minutes			
			Unheated control	70°C.	75°C.	80°C.
Nil (control)	mg.		cc.	cc.	cc.	cc.
	0	12	736	—	—	—
	1	6	738	—	—	—
	2	4	712	—	—	—
Serum protein	0	3	602	663	695	700
	1	2	712	760	742	695
	2	2	728	745	750	720
Casein	0	5	696	661	692	674
	1	4	702	682	718	674
	2	2	735	695	738	715
Lactose	0	3	597	626	645	642
	1	2	670	678	692	685
	2	2	690	702	685	695

¹ Quantity of potassium bromate per 100 g. of flour.

² Standard error of a single determination equals 27.6 cc.

teins, and lactose all depress loaf volume, with the last-mentioned having the greatest effect. In the absence of bromate, heat treatment improved the baking quality of the milk serum proteins considerably but had no effect on the baking quality of the other two fractions.

When bromate was used, the loaf volume of the bread baked with the milk serum protein fraction increased considerably especially when this fraction had not been heated before drying. Bromate also somewhat improved the baking results with lactose. Several investigators have noted that the inclusion of nonfat milk solids in a baking formula increases the bromate requirement of a dough (2, 9, 11). It now ap-

pears that the milk serum proteins and, to a small extent, lactose account for this effect.

The lower loaf volume of bread when the three milk fractions were added singly led to a series of baking tests designed to measure the effect on loaf volume of various combinations of the fractions (Table II). These were added to the dough formula in the amounts which would have been present had 6% of nonfat milk solids been used. A comparison of the loaf volumes in Tables I and II shows that the depressions caused by the various combinations are less than those which

TABLE II

EFFECT OF HEAT TREATMENT OF MILK CONSTITUENTS ON THE LOAF VOLUME OF BREAD WHEN BAKED IN COMBINATION AT VARIOUS BROMATE LEVELS
(Fractions heat treated before combining and added in amounts per loaf equal to those present in 6.0 g. of nonfat milk solids)

Milk constituent	Potassium bromate ¹	No. of replicates	Mean loaf volume ² for samples heated for 30 minutes			
			Unheated control	70°C.	75°C.	80°C.
	mg.		cc.	cc.	cc.	cc.
Casein + serum protein	0	4	601	646	642	618
	1	3	615	672	650	583
	2	2	635	708	680	635
Lactose + serum protein	0	3	582	640	642	672
	1	2	670	732	742	698
	2	2	688	680	702	665
Casein + lactose	0	3	662	662	683	642
	1	2	712	685	665	655
	2	2	708	695	688	680
Casein + lactose + serum protein	0	5	595	647	641	623
	1	2	645	658	672	645
	2	2	645	680	685	665

¹ Quantity of potassium bromate per 100 g. of flour.

² Standard error of a single determination equals 24.5 cc.

would be expected from the additive effects of the individual fractions. Whenever the milk serum proteins are present, the improving effect of heat treatment is apparent, again demonstrating the importance of this fraction in the heat-induced reaction.

Since the fractions were heat treated after their isolation, the question arises whether the conclusions concerning the differential effects of the treatment on loaf volume are applicable to separated milk itself. Rowland has shown that heat treatment of milk at 80°C. for 30 minutes results in the precipitation of most of the serum proteins

along with the casein upon subsequent acidification (10). An interaction between the serum proteins and casein may possibly occur upon the heat treatment of milk which might result in a different effect on baking properties than that obtained in these and other studies (6) where the heat treatments were applied to various fractions after their isolation.

To investigate this possibility, casein and the milk serum protein fraction which had been prepared in the manner already described were combined to yield a solution containing the same concentrations of these proteins as separated milk; aliquots of this solution were heat

TABLE III

EFFECT OF MILK CONSTITUENTS HEATED TOGETHER COMPARED TO HEAT-TREATED NONFAT MILK SOLIDS ON THE LOAF VOLUME OF BREAD

(Amount of each fraction per loaf equals that present in 6 g. of nonfat milk solids)

Material heat treated	Potassium bromate ¹	No. of replicates	Mean loaf volume ² for samples heated for 30 minutes			
			Unheated control	70°C	75°C	80°C
Control (No milk constituents)	mg		cc	cc.	cc.	cc.
	0	12	736	—	—	—
	1	6	738	—	—	—
Casein + serum protein	0	2	602	702	705	710
	1	2	618	705	708	698
Casein + lactose + serum protein	0	2	588	648	658	678
	1	2	610	660	688	672
Skim milk	0	2	570	582	678	672
	1	2	588	590	638	648

¹ Quantity of potassium bromate per 100 g. of flour.

² Standard error of a single determination equals 24.3 cc.

treated at different temperatures, and the solids isolated. Similarly, casein, serum proteins, and lactose were heated in combination so that the quality of this synthetic milk after drying could be compared with that of nonfat milk solids which had been prepared directly from skim milk subjected to similar heat treatments. The loaf volumes are summarized in Table III.

Heat treatment markedly improved the baking quality of the combinations, and any interaction of casein and serum protein during heating does not interfere with the baking behavior of the latter fraction. In fact, when lactose was also included, the baking results for the mixture were so similar to those for nonfat milk solids that it may

be concluded that these three fractions fully account for the influence of nonfat milk solids in breadmaking.

With the flour and baking procedure employed in these tests, the inclusion of nonfat milk solids in the baking formula lowers the loaf volume. This is contrary to the majority of reports in the literature. However, most investigators have used shortening in their formulas, and E. G. Bayfield⁷ has found that improvement in loaf volume is only obtained upon the addition of milk solids if the formula also contains shortening. This observation has been confirmed by the authors, but the use of shortening did not increase the relative differences in loaf volume obtained with fractions which were subjected to different heat treatments. In view of this and the desirability of limiting the number of variables in the baking test to the essential minima, shortening has not been used in these studies.

The finding that the baking improvement obtained by heat treating separated milk is associated with the serum protein fraction is in agreement with the findings of Harland, Ashworth, and Golding (6) which were published while the studies reported here were in progress. They found that the baking properties of dialyzed acid and rennet "wheys" prepared from skim milk were improved by heat treatment, whereas casein prepared by the acid method was not benefited; also, heat treatment had practically no effect on the dialyzable portion of the milk.

Physical Dough Tests. Typical farinograms and extensograms illustrating the effect of heat treatment of skim milk and its fractions on the physical properties of doughs are reproduced in Fig. 1. Curves are included for doughs made without milk and also for commercially prepared nonfat milk solids known to be of good and poor quality.

The farinograms show that the unheated milk serum proteins caused marked softening of dough upon extended mixing, a property which was greatly reduced by heat treatment; casein also had a slight softening action. The similarity between the curves for unheated serum proteins and laboratory-dried nonfat milk solids prepared from unheated separated milk and those for these preparations when heat treated is very striking. There was little difference between the plastic properties of the control dough and of those made with nonfat milk solids of high quality.

The extensograms for doughs made with nonfat milk solids prepared from unheated separated milk and of unheated serum proteins are characterized by very great extensibility and low resistance to extension. Heat treatment, however, yields extensograms which are very similar

⁷Private communication concerning unpublished studies at Kansas State College, Manhattan, Kansas. Present address Standard Milling Company, Chicago, Illinois.

to those for the dough made without milk. The differences between the extensograms for nonfat milk solids of good and poor baking quality are more striking than between the corresponding farinograms and it would appear that the extensograph would serve as a useful index of the quality of nonfat milk solids for breadmaking.

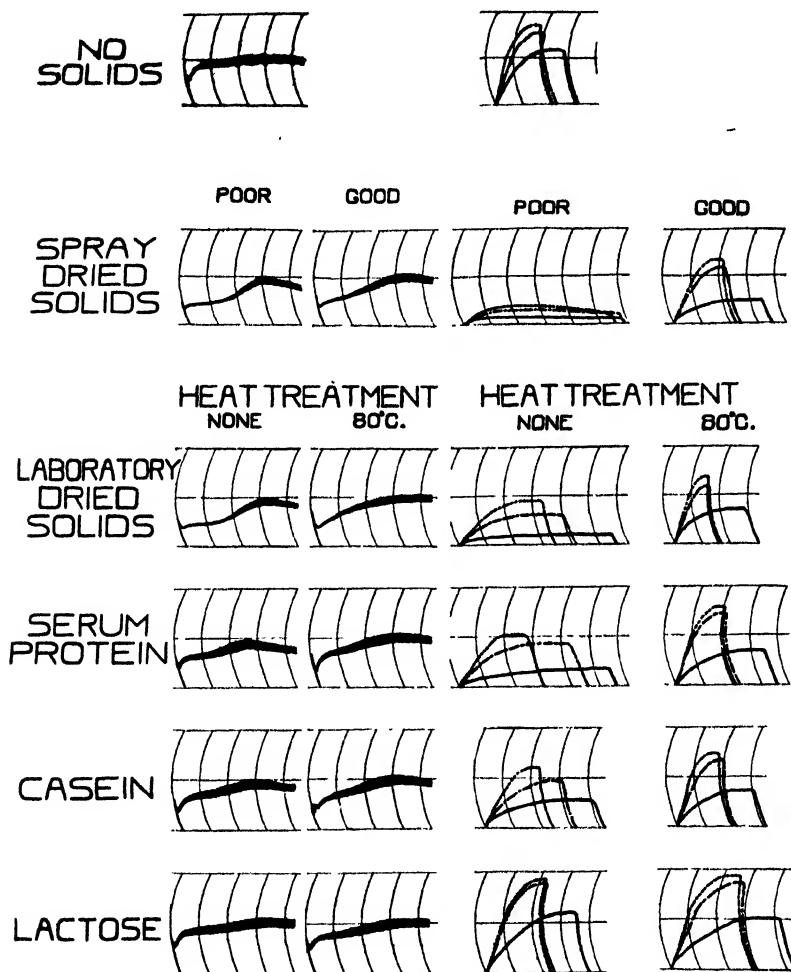


Fig. 1. The effect of nonfat milk solids and fractions thereof on the physical properties of a salt-water dough as measured by the farinograph and extensograph. The extensograms for a rest period of 45 minutes are shown by a solid line; those for a 90-minute rest period by a dot-dash line; and those for a 135-minute rest period by a dot-dot line.

Relation of Time and Temperature of Heating to Baking Quality.
The loaf volumes obtained for doughs containing 12% of nonfat milk solids prepared from defatted milk which had been preheated at vari-

ous temperatures and times are shown in Fig. 2. For the flash-heat treatments and that conducted for 30 minutes, the improvement in loaf volume to a maximum occurred within a rather narrow temperature range; further increases in temperature had little if any effect on loaf volume. Unfortunately, the equipment available for the 7-minute heat treatment did not have sufficient capacity to obtain temperatures higher than 85°C., but it appears from the other curves that a slightly higher temperature may be necessary to obtain maximum improvement. The standard error of the mean values recorded in Fig. 2 is 10.5 cc. and the apparent decreases in loaf volume in two of the series when the milks were preheated at high temperatures are not statistically significant. The minimum temperature required for maximum

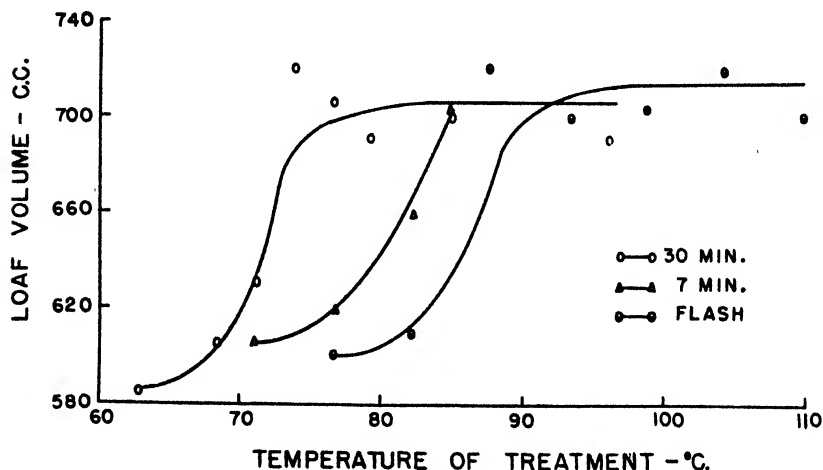


Fig. 2. Effect of time and temperature of preheat treatment on the baking properties of nonfat milk solids.

loaf volume increased with a decrease in heating time, being 73°C. for 30 minutes, about 85°C. for 7 minutes, and 92°C. for the flash method (about one minute). These treatments may be considered as the minimum necessary in the commercial production of nonfat milk solids of good baking quality. Higher temperatures might be desirable provided browning of the solids does not occur.

Discussion

These researches fully confirm the belief of Stamberg and Bailey (13) and of Harland, Ashworth, and Golding (6) that the improvement in the quality of separated milk for breadmaking which is brought about by heat treatment is associated with biochemical changes in the serum proteins. In addition, it has been found that casein and lactose

also lower loaf volume, an influence that is independent of the heat treatment. The action of casein can be explained as being due to the formation of a complex with gluten, thereby lowering its strength, but the role of lactose is not so readily explained. When this sugar was added to doughs in an amount equivalent to that present in 12% of nonfat milk solids no definite evidence was obtained that it had any influence on either gas production or gas retention. The farinograph tests indicate that lactose slightly increases dough consistency and that its harmful effects in baking might have been less pronounced if higher absorptions had been used.

Nonfat milk solids contain 36% casein, 50% lactose, and 7.5% serum proteins. It is significant that the serum proteins, which are present in such a low percentage, have the greatest influence on dough consistency, dough extensibility, and resistance to extension, and are responsible for the improvement in the baking quality of defatted milk upon heat treatment. The fact that the milk serum proteins are much richer in sulfur than casein and cause a decrease in dough consistency similar to the addition of small quantities of sulfhydryl compounds, such as cysteine and glutathione (2, 3, 12, 15), led Stenberg and Bailey (13) to suggest that the dough-softening action of raw milk is caused by the sulfhydryl groups of cysteine and that upon heat treatment these groups are either oxidized to the more stable disulfide linkage or are masked by heat treatment. Their polarographic studies indicated that sulfhydryl groups disappeared when milk was heated. It is noteworthy that nonfat milk solids increase the quantity of potassium bromate required for optimum baking results. This was found to be true also for milk serum proteins.

The fact that improvement in the baking quality of defatted milk is a function of both the time and temperature of heating indicates that heat denaturation of the serum proteins is the fundamental biochemical reaction involved. Protein denaturation may cause a molecular rearrangement in which many of the sulfhydryl groups become less available for reaction, a mechanism which would have the same effect as oxidation. A study of the effect of heat on the biochemical properties of the milk serum proteins is in progress.

Acknowledgments

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AN EVALUATION OF CABINET FERMENTATION WITH COMMERCIAL SIZE SPONGES AND DOUGHS¹

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ABSTRACT

The fermentation of sponges in open troughs and in a three-trough capacity cabinet under actual commercial shop conditions was studied. This included tracing the expansion of the sponges, observing dough-room temperature, cabinet temperature, and sponge temperature increase, and the concentration of carbon dioxide in the atmosphere within the cabinet and above the cabinet sponges. An adaptation of the pressuremeter test was applied to compare the fermentation characteristics of cabinet and open-trough sponges, and crumb compression measurements were made on some of the resultant bread.

Traffic in and out of the type cabinet employed nullifies any attempt to "condition" the cabinet with respect to the carbon dioxide content of its atmosphere. The maximum carbon dioxide concentration attained in any part of the cabinet is approximately 20%. This was attained when a sponge time of 4 hours 30 minutes was employed. It is possible that, with a shorter sponge time, traffic would tend noticeably to reduce the carbon dioxide content.

When sponges at or near maximum rise completely fill the trough, the concentration of carbon dioxide over the sponge does not exceed 18%. If the size of the sponge is reduced so that at maximum rise, the sponge is still well below the top edge of the trough, the carbon dioxide concentration over the sponge builds up to as much as 37%.

Spring wheat flour apparently gives lower carbon dioxide concentrations than does hard winter wheat flour.

The cabinet and open-trough sponges rise at the same rate and to the same height. The progress of fermentation is apparently no different in the cabinet sponges from what it is in the open-trough sponges. Cabinet fermentation does not reduce sponge time, nor does it permit the use of greater absorption in the dough stage. The doughs from the cabinet sponges, for all practical purposes, machine no better than doughs from sponges fermented in open troughs.

The cabinet is effective in insulating the sponges against significant fluctuations in dough-room temperature. It usually maintains a slightly higher temperature than the dough room, and automatically provides sufficient humidity to prevent crusting of the sponges.

The bread resulting from the sponges fermented in the cabinet is, for practical purposes, no better in quality than the bread made from the sponges fermented in the open troughs, despite the higher carbon dioxide concentrations found over the cabinet sponges. The bread from the cabinet sponges is no softer than, and stales no differently from, the bread made from the open-trough sponges.

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In the February 28, 1942, issue of *Baker's Helper*, there was announced a method of fermenting sponges in a restricted space. This method has since been designated "cabinet fermentation." In the original article, it was represented that resort to cabinet fermentation reduced sponge time to about 2 hours 15 minutes; permitted the sponges to develop more expansion; resulted in 2% extra absorption in the dough; and, in general, yielded bread of better quality.

Since then, there has been much discussion of the process. Many of the observations reported have been lacking in objectivity, but at least three well-organized studies have been published thus far.

Johnson (3) by means of laboratory-scale baking tests found that the rate of expansion and the height of sponge were not affected by the atmospheric conditions involved in cabinet fermentation. He did report that, in an atmosphere containing between 15.5 to 25% carbon dioxide and with a sponge time of 3 hours, the bread produced was better than the control fermented in normal air. No effect on dough absorption was mentioned in this study; but it may be inferred that better handling qualities were noted in the doughs made from cabinet-fermented sponges.

Schoonover, Freilich, and Redfern (4) compared cabinet sponges and noncabinet sponges under varying conditions of dough-room temperature and humidity, sponge temperature, and time, as well as the amount of yeast. They concluded that cabinet fermentation is of no value under controlled conditions of temperature and humidity in which the room temperature is equal to, or greater than, the temperature of the sponge as it is discharged from the mixer. When the dough-room temperature was much lower than the starting temperature of the sponge, cabinet fermentation showed definite advantages over open-trough fermentation. They found that most of the doughs from the cabinet sponges machined better. It was concluded that the effectiveness of cabinet fermentation depends on the conditions under which it is used. The inference is that it functions best where advantage is taken of its insulating effect against low dough-room temperatures.

Sullivan and Richards (5) find a marked superiority in favor of the cabinet method. The points of superiority enumerated are: drier, more mellow doughs that machine better; 1 to 2% more absorption may be used in the dough stage; optimum mixing time is a few minutes less than with open-trough fermentation; a shorter sponge time may be employed; the bread has softer texture and thinner cell walls; and the cabinet provides a measure of temperature and humidity regulation.

The superiority of cabinet fermentation is attributed to the greater

concentration of carbon dioxide over the cabinet sponge during the period from the breaking of the sponge to the time of doughing. Effective results were obtained when the concentration of carbon dioxide during this period was not less than 20% and best results were experienced at much higher levels of carbon dioxide. It is suggested that the sponge is permeable to the gases above it and that since carbon dioxide is more soluble in water, the sponge is probably more permeable to carbon dioxide than to oxygen or nitrogen. Moreover, it is suggested that carbon dioxide exercises its effect specifically, upon either the protein or certain oxidizing enzyme systems, rather than upon anaerobiosis.

These studies were not available in 1944 when it was decided to evaluate cabinet fermentation on a commercial scale under actual shop conditions. To accomplish this, a series of qualitative tests were planned wherein, over a period of time, the doughs and bread resulting from open-trough and cabinet sponges were compared to controls representing the normal shop practice. In the test doughs, sponge time was checked at 30 minute increments from 4 hours 30 minutes to 3 hours. In a second series of tests, in an attempt to attain some objectivity, the rise of the sponges was measured. The carbon dioxide content of the atmosphere within the cabinet was measured at three different levels and above the sponge as fermentation progressed. An endeavor was made to note differences in fermentation characteristics through an adaptation of the gassing power test, and crumb compression measurements were made on the bread as well as observations on bread quality and characteristics. In addition, observations were made on dough-room temperature, cabinet temperature, and sponge temperature increase.

Materials and Methods

The work to be reported herein made use of a three-trough capacity cabinet constructed of plywood and illustrated in Fig. 1. Since the study extended over a considerable period of time, no attempt was made to work with a single lot of flour. However, unless otherwise specified, the flours used were milled from hard winter wheat and conformed to the following specifications:

% Protein (14% moisture basis).....	10.5% to 11.0%,
% Ash (14% moisture basis).....	0.42% maximum,
Gassing power (6 hours).....	400-450 mm. Hg.,

where gassing power is determined by the method described in *Cereal Laboratory Methods* (1).



Fig. 1. Experimental cabinet installation

A typical commercial formula making use of a 60% sponge was employed. The normal sponge time employed in the shop was 5 hours. The sponges were mixed 5-6 minutes and were set at 78°-79°F. (25.5°-26.1°C.). The doughs were mixed in accordance with the requirement of the flour (10 to 14 minutes), were set at 78°-80°F. (25.5°-26.7°C.), and were subjected to 20 minutes' floor time. An attempt was made to maintain the dough room at 80°F. (26.7°C.), although experience demonstrated that it varied between 79° and 85°F. (26.1° and 29.4°C.). The doughs were handled over the usual make-up machinery with a minimum of intermediate proof. Conditions maintained in the steam-box were 98°-100°F. (36.7°-37.8°C.), with relative humidity at 90% or higher. The bread was baked with a little steam in the oven and in accordance with commercial practices as to temperature and time. A 22-ounce plain-top loaf was made throughout this study.

The work was carried out in two stages. The first consisted of a series of qualitative tests which provided a background of experience for designing the second series of tests in which an attempt was made to measure objectively some of the factors involved.

Qualitative Tests. The technique employed in the qualitative

tests was to set three cabinet sponges and two open-trough control sponges each day. In order to "condition" the cabinet, it was filled with three sponges which were not involved in the test and which were so scheduled that, as each one was ready for remixing, a test cabinet sponge took its place in the cabinet. For the first series, the sponge time for the cabinet sponges was arbitrarily established as 4 hours 30 minutes. One of the open-trough control sponges was given the same sponge time and the other was run on the regular shop schedule of 5 hours. Then, 4-hour sponges in the cabinet were compared with a 4-hour sponge and a 5-hour sponge on the floor. The technique was repeated, each time reducing the sponge time of the cabinet sponges by 30 minutes and comparing them with controls in open-troughs, one at 5 hours and the other comparable in sponge time to the cabinet sponges. This process was continued until a sponge time of 3 hours had been tested.

Objective Tests. The second phase of the study consisted of three tests making use of hard winter wheat flours conforming to the specifications already described. The tests were so spaced as to include hard winter wheat flours from the end of the 1943 crop, the forepart of the 1944 crop, and the first third of the main body of the 1944 crop.

In these tests the rise of the sponges was measured objectively. The carbon dioxide content of the atmosphere within the cabinet was measured at three different levels as well as over the sponges as fermentation progressed. An attempt was made to note differences in fermentation characteristics through an adaptation of the gassing power test, and crumb compression measurements were made on the bread.

The device which was improvised to measure the rise of the sponges is illustrated in Fig. 2. Essentially it consists of a 12-inch by 12-inch metal plate *A* attached to a square rod *B* so mounted and guided as to permit only vertical movement. The system is so counterbalanced that the plate can follow any recession of the sponge, but cannot exert sufficient pressure to cause it to settle into the sponge. An arbitrarily chosen scale *C* is mounted adjacent to rod *B*. On the rod is an adjustable pointer *D* to facilitate the measurement of sponge movement. When a sponge is set, the "riseometer" is mounted over the sponge in the approximate center of the trough. The plate is lowered until it is just in contact with the sponge and then the adjustable pointer is set at "3." As fermentation progresses, the operator notes the scale readings at definite intervals of time. From these data can be constructed total-rise graphs or rate-of-rise graphs which are not dissimilar in their general contours to the analogous graphs that are



Fig. 2. Riseometer.

obtained from the results of gassing power tests, at least up to the time the sponge reaches its maximum height.

An Orsat apparatus was employed to determine the concentration of carbon dioxide at three levels in the cabinet and at a point about one inch above the surfaces of the cabinet sponges. The three levels in the cabinet corresponded, respectively, to heights 6 inches above the floor, about one-half that of the trough, and the top edge of the trough. Determinations were made as frequently as facilities and personnel available permitted over the period of time beginning with the entry of the first test sponge into, and the exit of the last test sponge from,

the cabinet which was not compartmentalized. No attempt was made to accumulate similar data for the dough room or for the space over the sponges therein, because the primary interest was in the cabinet conditions.

As another means of gaining insight into the progress of fermentation in cabinet sponges as compared with sponges fermented in open troughs in the dough room, aliquots of sponge were taken after fermentation had progressed 1, 2, 3, and 4 hours, were transferred to pressuremeters, and were then fermented at 30°C. for 6 hours. The hourly pressure readings were plotted and the contours and slopes of the various graphs, grouped according to the stage of sponge time, were compared.

The bread resulting from these tests was measured for crumb compressibility over a holding period of 5 days. The machine utilized was made to the specifications of Combs (2) and compressibility was expressed as the weight of mercury required to compress a sample one inch thick, an amount corresponding to 0.35 inch.

Results and Discussion

Qualitative Tests. One of the first things observed was that the cabinet sponges differed in appearance from the sponges on the floor. They did not crust and the top surface remained soft and moist. The gas cells were smaller and more uniformly distributed. The sponges from the cabinet felt drier when manipulated. However, the resultant bread was quite uniform in quality whether it was made from a cabinet sponge, an open-trough sponge of comparable time, or a 5-hour open-trough sponge. Nor did the make-up personnel note any differences in machining properties between any of the test doughs.

As a result of the observation on the appearance and the "feel" of the cabinet sponges, the test was repeated with one factor changed: the doughs from the cabinet sponges were given an increment of 1% extra absorption. All dough mixing times were kept the same. Again no advantages in bread quality could be noted, and it was concluded that the cabinet-method doughs do not possess extra absorption capacity. These doughs, however, machined satisfactorily.

Until a 3-hour sponge time was worked with, it was found that all of the bread produced in these tests was of comparable quality. The 3-hour sponges from the cabinet produced bread no better than that produced by the 3-hour sponge fermented in an open trough. Compared with the 5-hour sponge fermented in an open trough, the 3-hour sponges (from cabinet or open trough) produced bread slightly inferior in quality—not as good dough-to-dough or day-to-day uniformity.

As the result of this first set of experiments it was tentatively concluded that cabinet fermentation is not effective in reducing sponge time, increasing the absorption of the dough, improving the machining properties of the dough, or raising the quality of the bread. It was felt, however, that the cabinet does minimize the effect of temperature fluctuation in the dough room and effectively and economically provides adequate humidity to prevent sponges from crusting.

Objective Tests. In general, the practices and conditions already described were followed except that a sponge time of 4 hours 30 minutes was used in all tests. After the cabinet had been "conditioned," two normal-size sponges and one half-size sponge were placed in it. These were compared with a normal size and a half-size sponge fermented outside the cabinet in open troughs. Since the quantity of sponge (570 pounds) normally used in the shop where this work was done completely fills the trough at full rise, thus permitting any "blanket" of carbon dioxide to cascade off, the half-size sponge was made in the cabinet to insure that a high concentration of carbon dioxide would be retained over it.

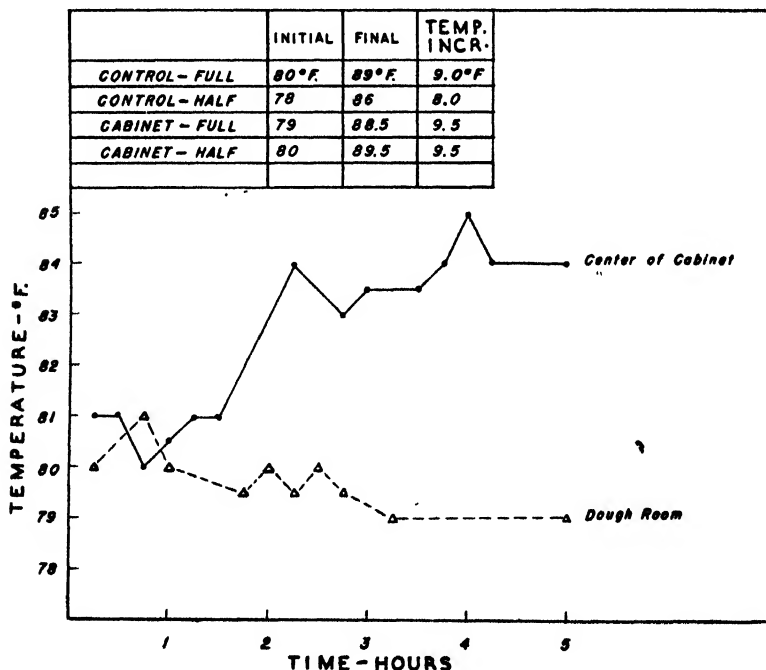


Fig. 3. Progression of temperature in the cabinet and in the dough room over the test period with lights operating in the cabinet, along with the temperature characteristics of the test sponges and the temperature increase of the sponges. Test 1, utilizing a hard winter wheat flour (1943 crop), half- and full-size sponges in room and cabinet, and a sponge period of 4 hours 30 minutes.

Tests 1 and 2 were identical except that the flour used, while conforming to the specifications already given, came from different shipments and different mills. In the cabinet were two full-size and one half-size sponges, while in the dough room in open troughs were a full-size and a half-size sponge. All were fermented 4 hours 30 minutes. Unless otherwise specified, all other conditions fell within the ranges already described.

Test 3 made use of a third lot of hard winter wheat flour, as already specified, and a spring wheat flour, generously furnished by Dr. Betty Sullivan of the Russell-Miller Milling Company. It analyzed 0.40% ash and 11.5% protein. Since the quantity of this flour was limited, it was not possible to mix it any more than was the hard winter wheat flour; and the absorption applied was on the conservative side of the flour's requirement. In the "conditioned" cabinet were fermented a full-size hard winter wheat flour sponge, a full-size spring wheat flour sponge, and a half-size spring wheat flour sponge. In the dough room were fermented in open troughs a full-size hard winter wheat flour sponge, a full-size spring wheat flour sponge, and, in a covered trough,

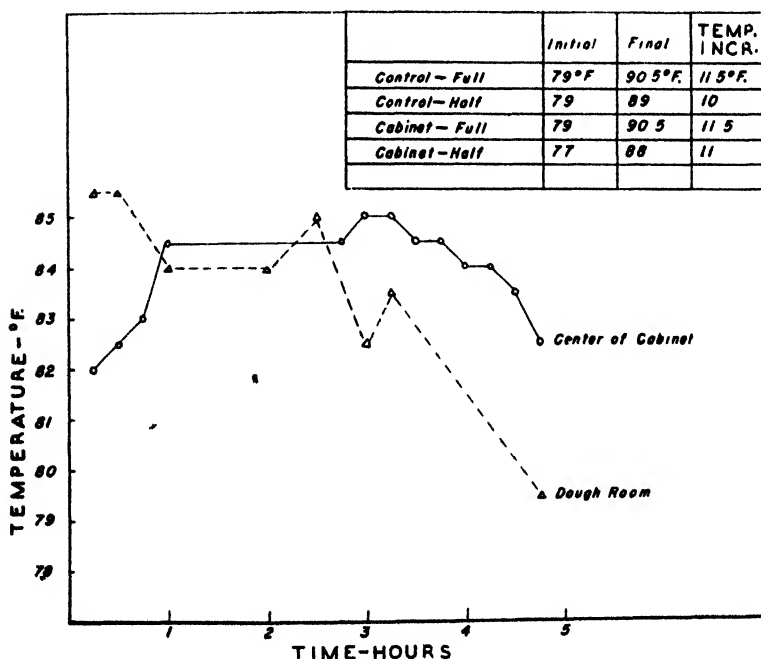


Fig. 4. Progression of temperature in the cabinet and in the dough room over the test period without lights operating in the cabinet, along with the temperature characteristics of the test sponges and the temperature increase of the sponges. Test 2, utilizing a hard winter wheat flour (early 1944 crop), half- and full-size sponges in room and cabinet, and a sponge period of 4 hours 30 minutes.

a full-size spring wheat flour sponge. All sponge times were 4 hours 30 minutes and, unless otherwise specified, the shop conditions were as previously noted.

Figs. 3, 4, and 5 record the march of temperature over the three test periods in the cabinet and in the dough room itself. In addition, they show the temperature increase of the sponges.

Fig. 3 covers test 1, during which the lights in the cabinet were permitted to operate. As a consequence, the cabinet temperature built up, despite a tendency for the dough-room temperature to diminish. This fact emphasizes the insulating effect of the cabinet and suggests that, in emergencies, the burning of a few incandescent lamps would be effective in raising the cabinet temperature. The increases in temperature of the corresponding sponges in open troughs and in the cabinet are little different and can be accounted for readily by environmental temperatures and relative exposure of the sponges thereto.

In test 2 (Fig. 4) the lights were not used. In general, the cabinet tended to follow the temperature trend of the room; a lag in its re-

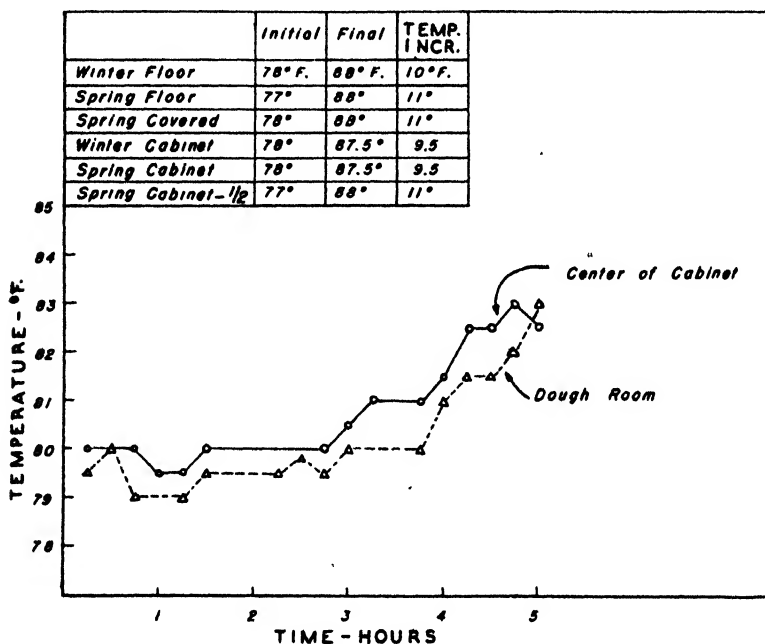


Fig. 5. Progression of temperature in the cabinet and in the dough room over the test period without lights operating in the cabinet, along with the temperature characteristics of the test sponges and the temperature increase of the sponges. Test 3, utilizing a hard winter wheat flour (1944 crop) and a spring wheat flour, with sponges in room and cabinet as described, and a sponge period of 4 hours 30 minutes.

sponse is apparent. The full sponges, whether in the cabinet or in an open trough, experienced the same temperature increase. The temperature increases in the half-size sponges fermented in the cabinet and in an open trough are very similar. Any differences can be accounted for by the difference in the environmental temperatures in which they resided.

Fig. 5 illustrates the degree of parallelism in temperature between the room and the cabinet. There is a general tendency for the cabinet temperature to be slightly higher than that of the room. The sponges made from hard winter wheat flour registered no practical difference in temperature increase. Among the full sponges made from spring wheat flour, that in the open trough and that in the covered trough developed the same temperature increase. The behavior of the full-size spring wheat flour sponge in the cabinet is difficult to explain, but it may illustrate the limitations under which sponge temperatures are measured under shop conditions. The temperature increase for the half-size spring wheat flour sponge is somewhat more in line with the general picture.

Figs. 6, 7, and 8 depict the concentrations of carbon dioxide in the cabinet over the test period and at the three levels checked. Points A, B, and C indicate the removal of the sponges used to "condition"

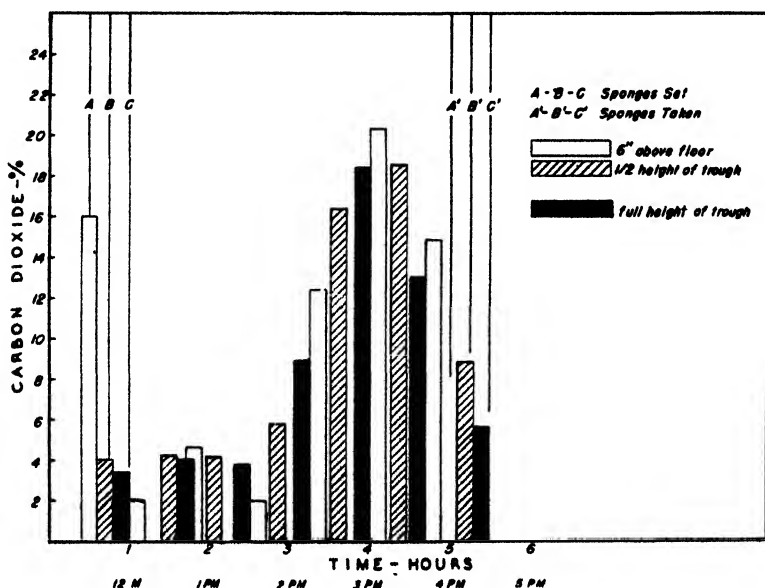


Fig. 6. Concentration of carbon dioxide in the cabinet at three levels at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 1, utilizing a hard winter wheat flour (1943 crop) and a sponge period of 4 hours 30 minutes.

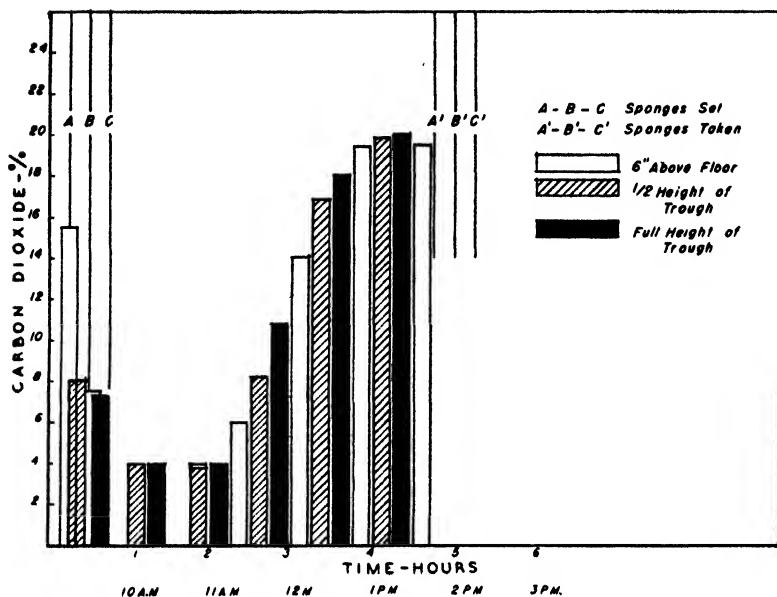


Fig. 7. Concentration of carbon dioxide in the cabinet at three levels at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 2, utilizing a hard winter wheat flour (early 1944 crop) and a sponge period of 4 hours 30 minutes.

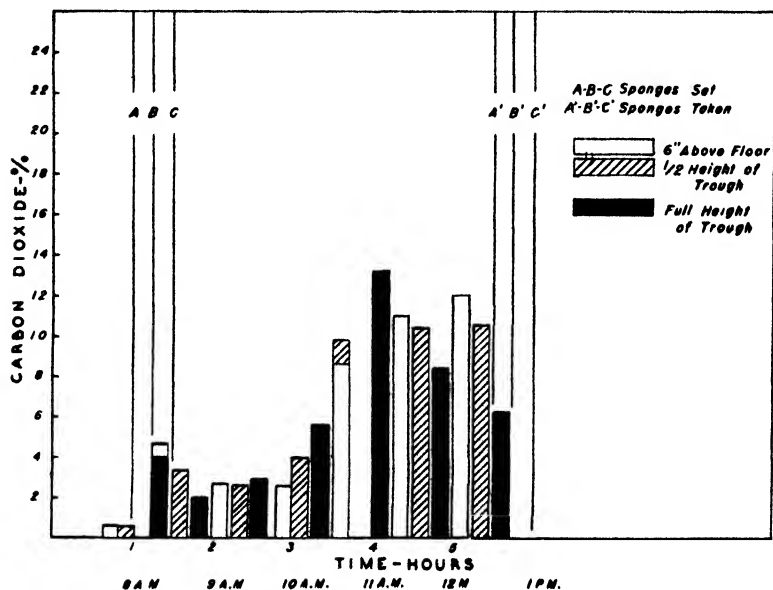


Fig. 8. Concentration of carbon dioxide in the cabinet at three levels at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 3, utilizing a hard winter wheat flour (1944 crop), a spring wheat flour, and a sponge period of 4 hours 30 minutes.

the cabinet and the introduction of the test sponges. Points A^1 , B^1 , and C^1 are the times at which the test sponges were removed, thus progressively emptying the cabinet.

Figs. 6 and 7 have several points of similarity and suggest that the carbon dioxide concentration built up during the "conditioning" period is rather effectively diluted by the traffic into and out of the cabinet. Then the carbon dioxide concentration builds up gradually to about 18 to 20% relatively late in the sponge time. There is some evidence that cascading from the full sponges is experienced and that stratification develops, although this is not pronounced. Finally, traffic causes the carbon dioxide concentration in the cabinet to diminish markedly.

Fig. 8 showing the results for two sponges made from spring wheat flour and one sponge made from hard winter wheat flour, reflects approximately the same sequence of events, except that the maximum concentration of carbon dioxide is only 12 to 14%. Whether this is characteristic for the spring wheat flour used or the result of an unknown experimental error could not be determined.

The concentration of carbon dioxide approximately one inch above

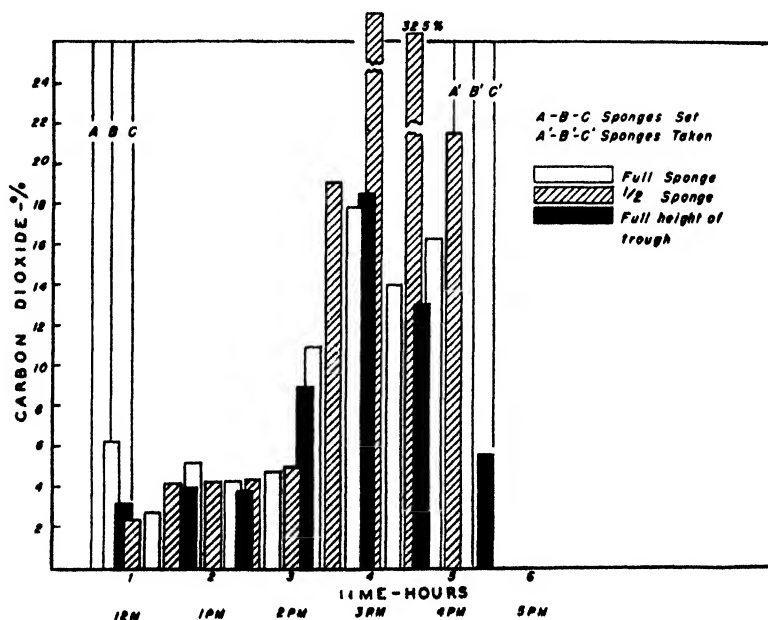


Fig. 9. Concentration of carbon dioxide in atmosphere within cabinet, one inch above the test cabinet sponges, and at a level corresponding to the height of the trough at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 1, utilizing a hard winter wheat flour (1943 crop) and a sponge period of 4 hours 30 minutes.

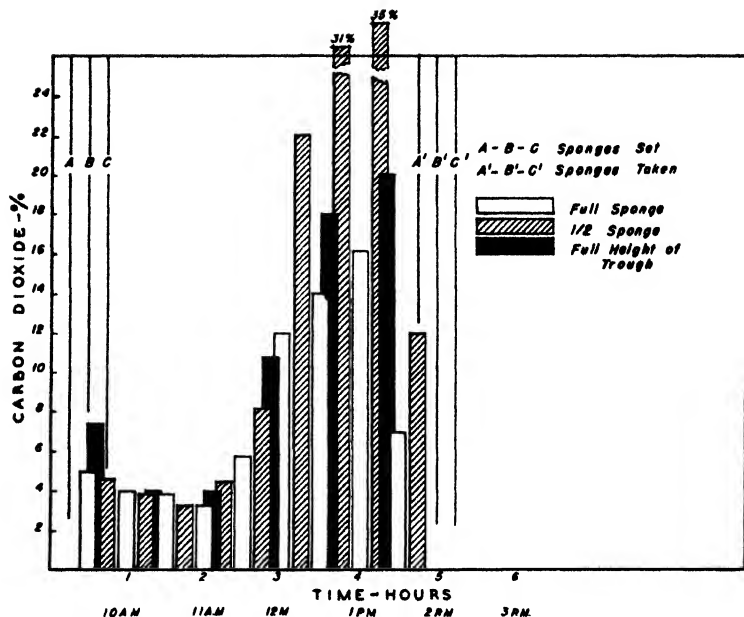


Fig. 10. Concentration of carbon dioxide in atmosphere within cabinet, one inch above the test cabinet sponges, and at a level corresponding to the height of the trough at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 2, utilizing a hard winter wheat flour (early 1944 crop) and a sponge period of 4 hours 30 minutes.

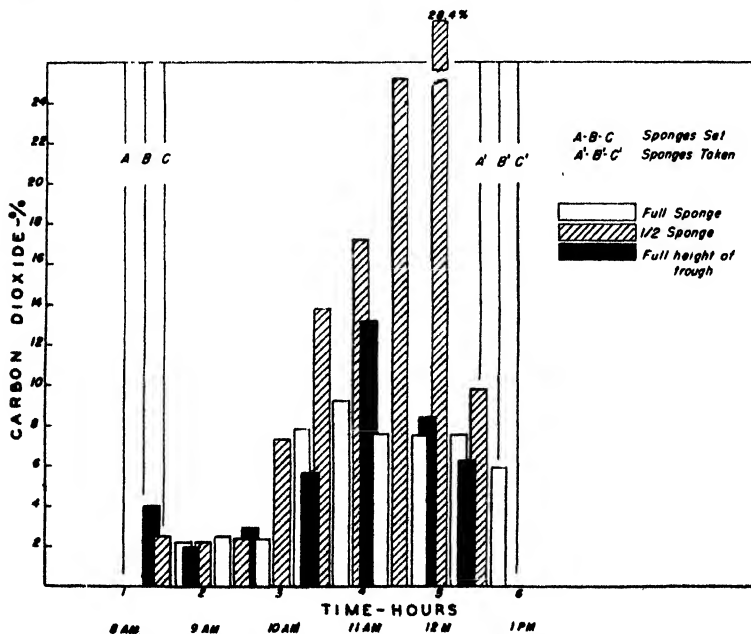


Fig. 11. Concentration of carbon dioxide in atmosphere within cabinet, one inch above the test cabinet sponges, and at a level corresponding to the height of the trough at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 3, utilizing a spring wheat flour and a sponge period of 4 hours 30 minutes.

the surface of the sponge is shown in Figs. 9, 10, and 11. For example, Fig. 9 brings out that the full-size sponge and the half-size sponge each develop a maximum concentration of carbon dioxide within about $3\frac{1}{4}$ hours; but that the level attained over the full sponge was almost 18% as compared with 37% over the half sponge.

In test 2 illustrated by Fig. 10, the maxima appear within approximately $3\frac{3}{4}$ hours. That over the full sponge is slightly more than 16% compared with 35% over the half sponge.

In test 3, which involved the use of spring wheat flour, the progression of carbon dioxide concentration over the cabinet sponges is depicted in Fig. 11. The maximum concentration over the full sponge would seem to come within about $2\frac{1}{4}$ hours, which is erratic when compared with the appearance of the maximum concentration

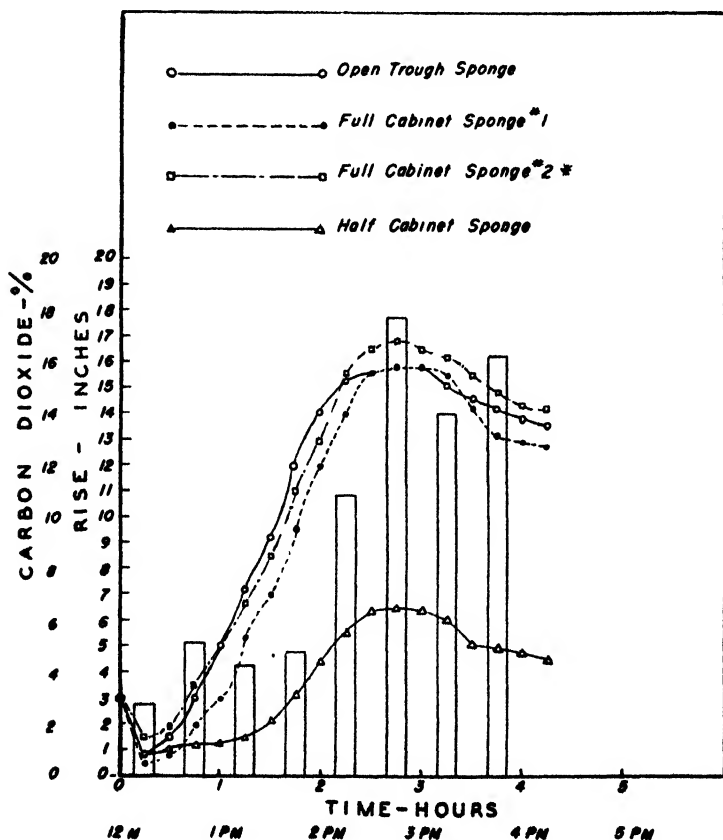


Fig. 12. Progression of rise of test cabinet sponges in comparison with a comparable open-trough sponge and in relation to concentration of carbon dioxide, one inch above cabinet sponge number 2. Test 1, utilizing a hard winter wheat flour (1943 crop) and a sponge period of 4 hours 30 minutes.

over the half sponge at about $3\frac{3}{4}$ hours. The only plausible explanation for this is that the appearance of the true maximum for the full sponge is obscured by the cascading of the carbon dioxide off the surface of this sponge, which stood high in the trough. Whatever the cause, the maximum carbon dioxide concentration over the full sponge was slightly in excess of 9% and over the half sponge, a little more than 28%. Both of these values are lower than those encountered in the two previous trials in which hard winter wheat flour was used.

The rise of the sponges is plotted in Figs. 12, 13, and 14, utilizing the "riseometer" already described. Fig. 12 shows graphs obtained for a full sponge in an open trough, two full sponges in the cabinet, and a half-size sponge in the cabinet. The two full cabinet sponges provide some idea of the degree of replication that can be attained

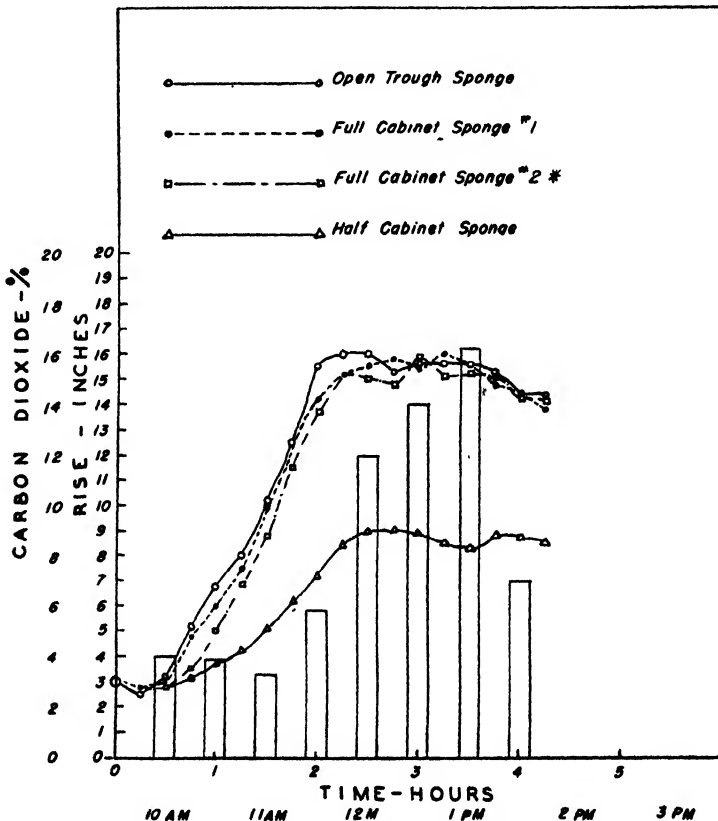


Fig. 13. Progression of rise of test cabinet sponges in comparison with a comparable open-trough sponge and in relation to concentration of carbon dioxide, one inch above cabinet sponge number 1. Test 2, utilizing a hard winter wheat flour, (early 1944 crop) and a sponge period of 4 hours 30 minute

with this device and hence assist in interpreting the results. With such provision as a guide, it is apparent that in test 1 the sponge fermented in the open trough did not behave any differently from the two cabinet sponges. The graph for the half-size cabinet sponge is given to show the parallelism existing between it and the full-size sponges in the cabinet, although it had over it, for a measurable period of time, a significantly higher concentration of carbon dioxide. The bar graph shows the progression of carbon dioxide concentration over the cabinet sponge designated number 2.

The results of test 2 are shown in Fig. 13. This experiment was conducted with a hard winter wheat flour differing in source and crop year from that used in test 1. It is evident that its fermentation char-

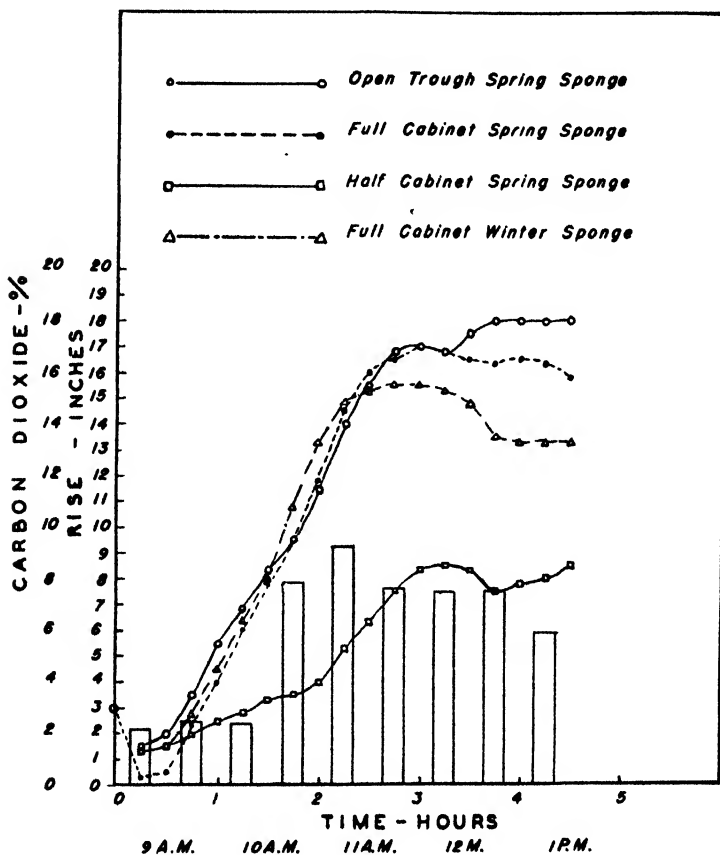


Fig. 14. Progression of rise of test cabinet sponges made with a spring wheat flour in comparison with a comparable open-trough sponge and a test cabinet sponge made with a hard winter wheat flour (1944 crop) and in relation to concentration of carbon dioxide, one inch above the full cabinet sponge made with spring wheat flour. Test 3, utilizing a sponge period of 4 hours 30 minutes.

acteristics vary from those of the flour used in test 1, yet the graphs for the full sponge in the open trough and the two full cabinet sponges practically coincide. Again there is marked parallelism between the half sponge in the cabinet and the full cabinet sponges which developed lower concentrations of carbon dioxide over their surfaces. Also, the bar graph illustrates the progression of carbon dioxide over cabinet sponge 2 in relation to its expansion in the trough.

The results of test 3 are illustrated in Fig. 14. It contains "rise-ometer" graphs for spring wheat flour sponges, including a full sponge in an open trough, a full sponge in the cabinet, and a half sponge in the cabinet. Besides, there is a graph for a full cabinet sponge made from a hard winter wheat flour.

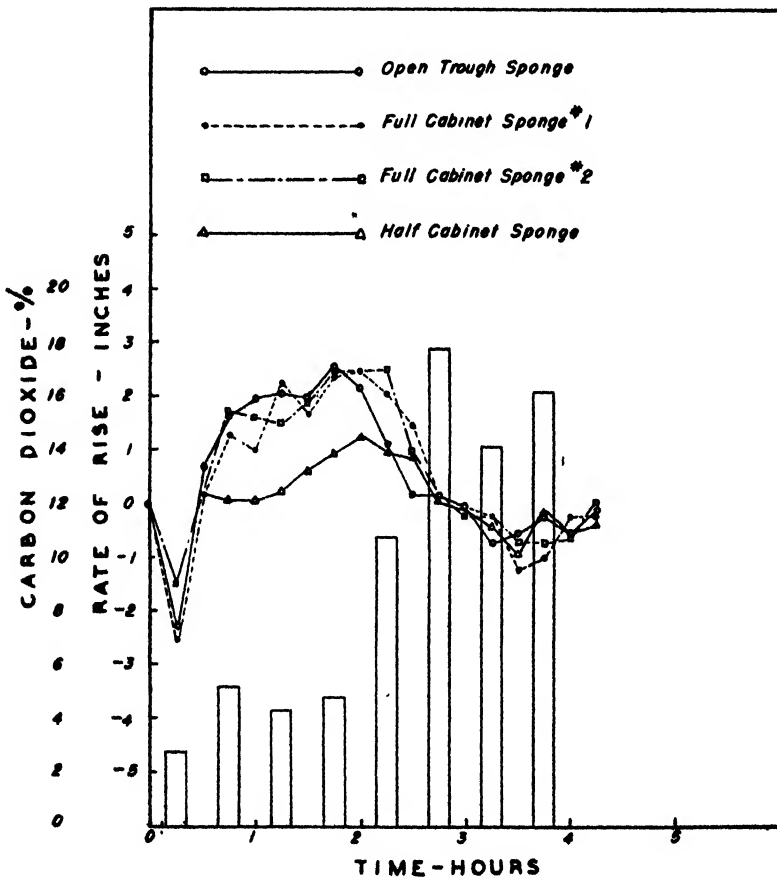


Fig. 15. Rate of rise of test cabinet sponges in comparison with a comparable open-trough sponge and in relation to concentration of carbon dioxide, one inch above cabinet sponge number 2. Test 1, utilizing a hard winter wheat flour (1943 crop) and a sponge period of 4 hours 30 minutes.

First of all, there is noted a characteristic difference between the hard winter wheat flour sponge and the two full-size spring wheat flour sponges, which is not contrary to expectations. There is great similarity between the open-trough and cabinet sponges utilizing spring wheat flour. The divergence apparent in the latter stage of the sponge period can be attributed to the crusting which was encountered on the sponge in the open trough. The graph for the half cabinet sponge reasonably parallels the comparable full-size sponge. The latter portion of the curve is in all probability influenced by the confinement of the sponge within the trough, whereas the full cabinet sponge to some extent overflowed its trough. The bar graph portrays

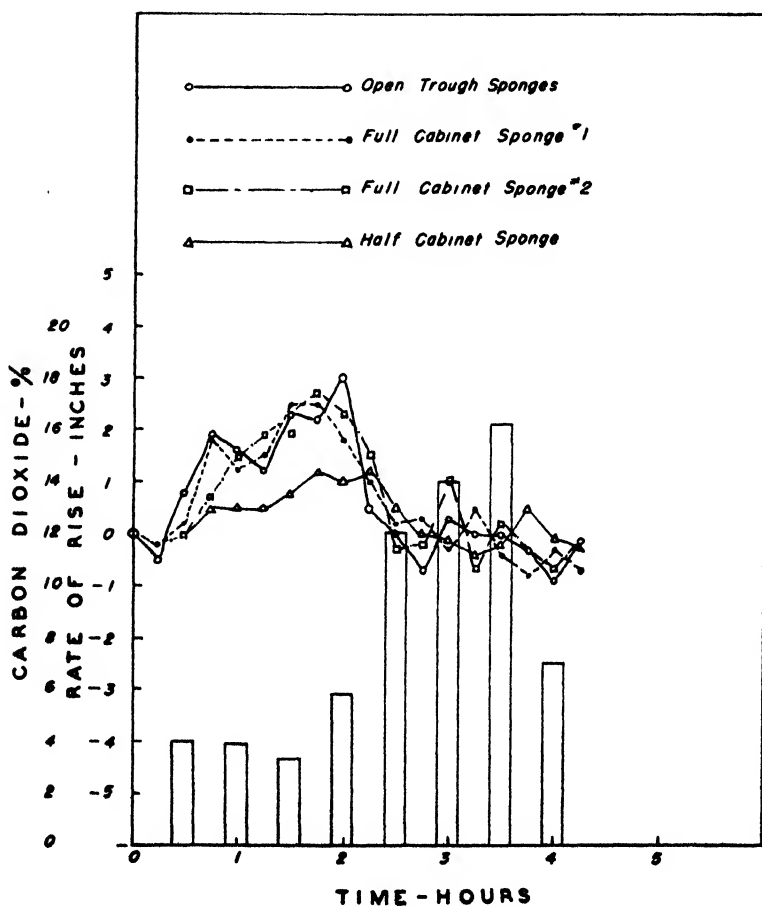


Fig. 16. Rate of rise of test cabinet sponges in comparison with a comparable open-trough sponge and in relation to concentration of carbon dioxide, one inch above cabinet sponge number 2. Test 2, utilizing a hard winter wheat flour (early 1944 crop) and a sponge period of 4 hours 30 minutes.

the march of carbon dioxide over the full cabinet sponge made with spring wheat flour. When related to its "riseometer" curve, and having in mind the comparable relationships to be observed in Figs. 12 and 13, there is reason to believe that the appearance of the maximum is premature and that the true maximum concentration of carbon dioxide was not realized, probably because the carbon dioxide cascaded from the sponge's surface.

Figs. 15, 16, and 17 show the data from the "riseometer" readings as rate curves and in this form depict a high degree of similarity between open-trough sponges and cabinet sponges in the progress of fermentation.

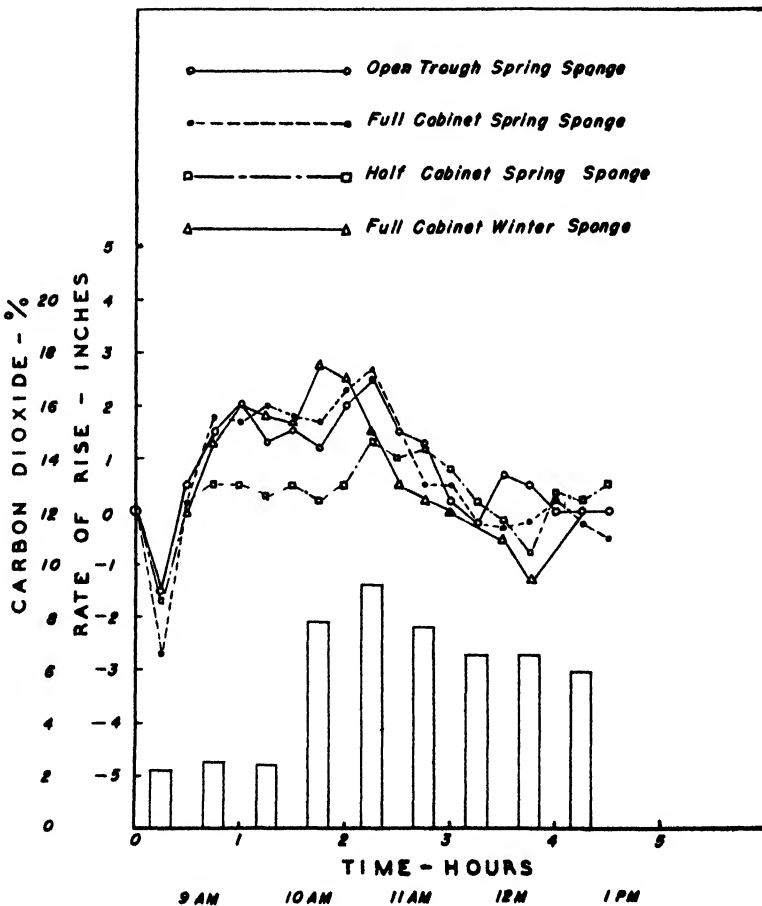


Fig. 17. Rate of rise of test cabinet sponges made with a spring wheat flour in comparison with a comparable open-trough sponge and a test cabinet sponge made with a hard winter wheat flour (1944 crop), and in relation to concentration of carbon dioxide, one inch above the full cabinet sponge made with spring wheat flour. Test 3, utilizing a sponge period of 4 hours 30 minutes.

The results from the adaptation of the gassing power tests are given in Figs. 18 and 19, and cover only tests 2 and 3, since its use was not conceived until test 1 had been carried out. Having in mind the conditions under which the tests were made in the shop, as well as the recognized limitations residing in pressuremeter tests with respect to concordance in absolute terms, it is concluded that these results portray no practical difference in fermentation between sponges

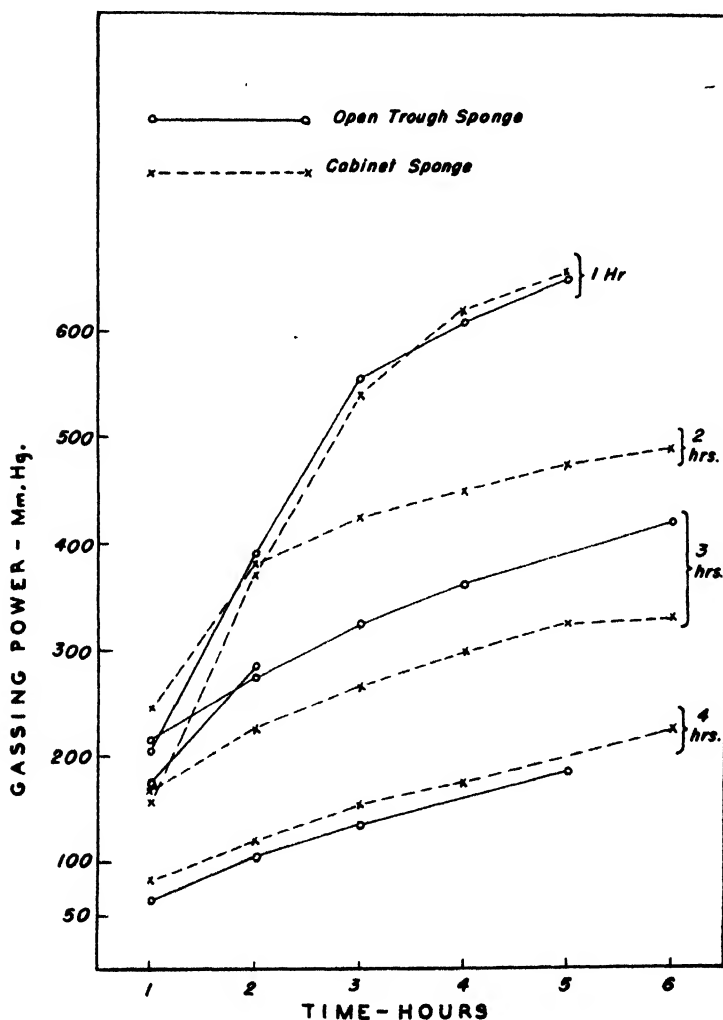


Fig. 18. Comparison of gassing power of open-trough and cabinet sponges, utilizing aliquots of sponge taken after 1, 2, 3, and 4 hours of fermentation and fermented for 6 hours in a pressuremeter at 30°C. Test 2, utilizing a hard winter wheat flour (early 1944 crop) and a sponge period of 4 hours 30 minutes.

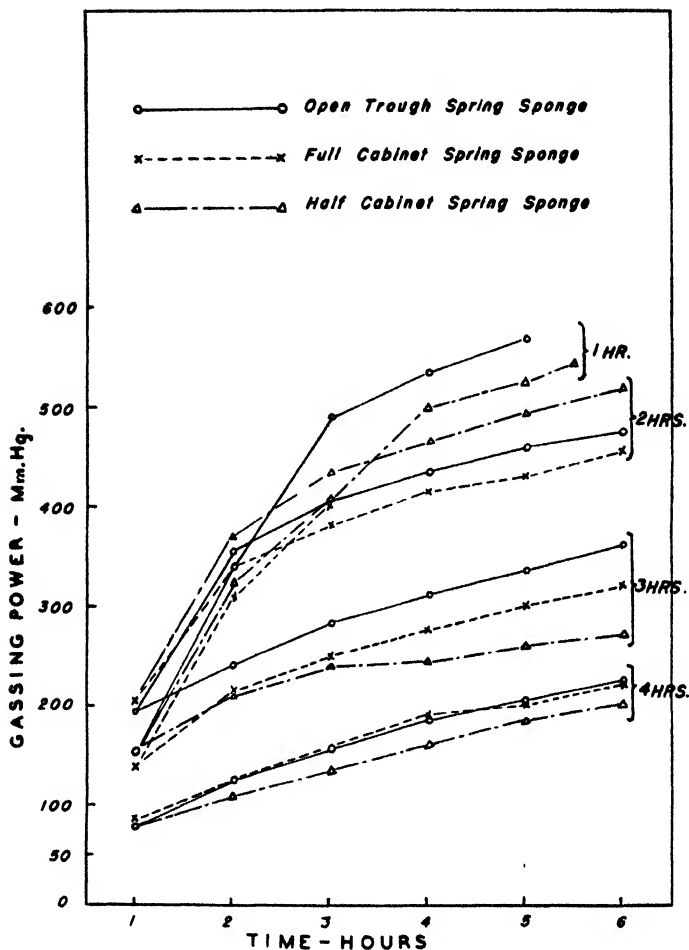


Fig. 19. Comparison of gassing power of an open-trough sponge with a full and half cabinet sponge, utilizing aliquots of sponge taken after 1, 2, 3, and 4 hours of fermentation and fermented for 6 hours in a pressuremeter at 30°C. Test 3, utilizing a spring wheat flour and a sponge period of 4 hours 30 minutes.

fermented in open troughs and sponges fermented in the cabinet. The sponge aliquots of equal fermentation time definitely group together, while the slopes of their pressuremeter curves reflect a high degree of parallelism.

As in the series of qualitative tests, no difference in the machinability of the doughs was noted by the personnel at the make-up units. Even the half-size sponges fermented in the cabinet, which had a significantly greater concentration of carbon dioxide over them, showed no differences.

An adequate number of sample loaves were taken from each test dough by the shop foreman and, on the day following the test, were examined by a panel of four. After careful examination, the consensus was that the quality and characteristics of the bread were comparable, regardless of whether the sponges were fermented in open troughs or in the cabinet, or whether the bread was made from half-size sponges fermented by the two methods.

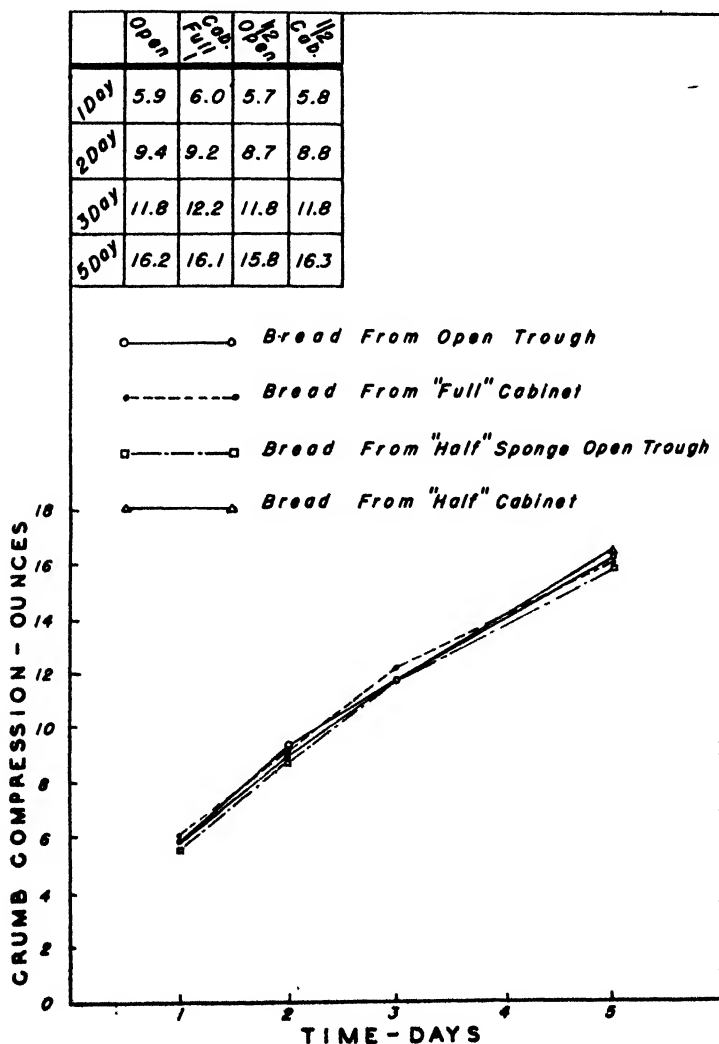


Fig. 20. Crumb compression measurements on bread from comparable open-trough and cabinet sponges made after 1, 2, 3, and 5 days holding at room temperature. Test 1, utilizing bread made from hard winter wheat flour (1943 crop).

The results of crumb-compression measurements on the bread resulting from the tests are shown in Figs. 20, 21, 22, and 23. Measurements were made 1, 2, 3, and 5 days after baking. The resultant staling curves for test 1 are given in Fig. 20. They coincide so closely as to leave little room to attribute any superiority in softness or keeping quality to the bread made from cabinet sponges. Fig. 21 shows that comparable results were obtained in test 2.

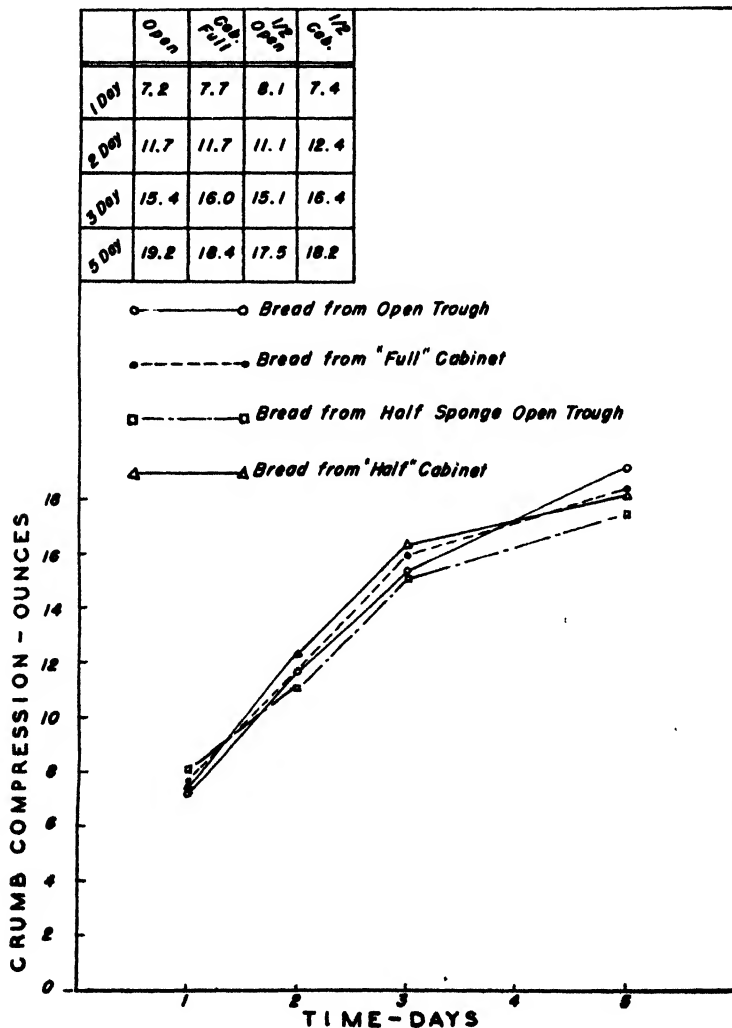


Fig. 21. Crumb compression measurements on bread from comparable open-trough and cabinet sponges made after 1, 2, 3, and 5 days holding at room temperature. Test 2, utilizing bread made from hard winter wheat flour (early 1944 crop).

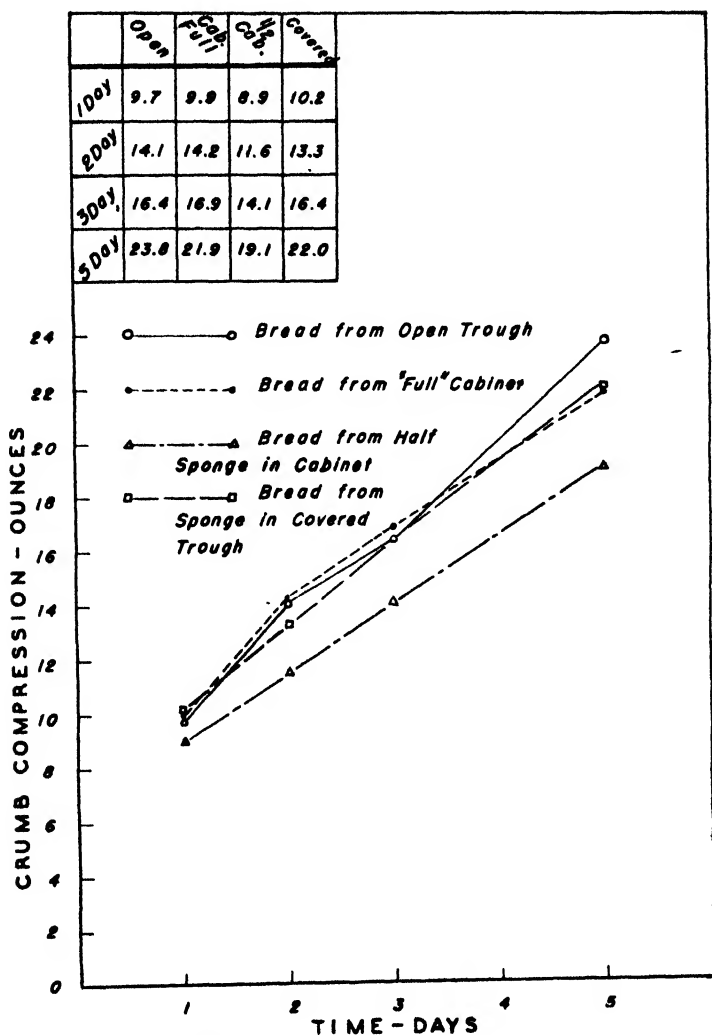


Fig. 22. Crumb compression measurements on bread from comparable open-trough and cabinet sponges made after 1, 2, 3, and 5 days holding at room temperature. Test 3, utilizing bread made from spring wheat flour.

Fig. 22 illustrates the crumb compression results obtained in test 3 with the bread made from spring wheat flour. Only the bread resulting from the half-size sponge fermented in the cabinet shows any advantage in softness. The samples from the other three sponges, open-trough sponge, covered-trough sponge, and full-size cabinet sponge, are quite comparable in softness.

Fig. 23 also applies to test 3. It contains the crumb compression

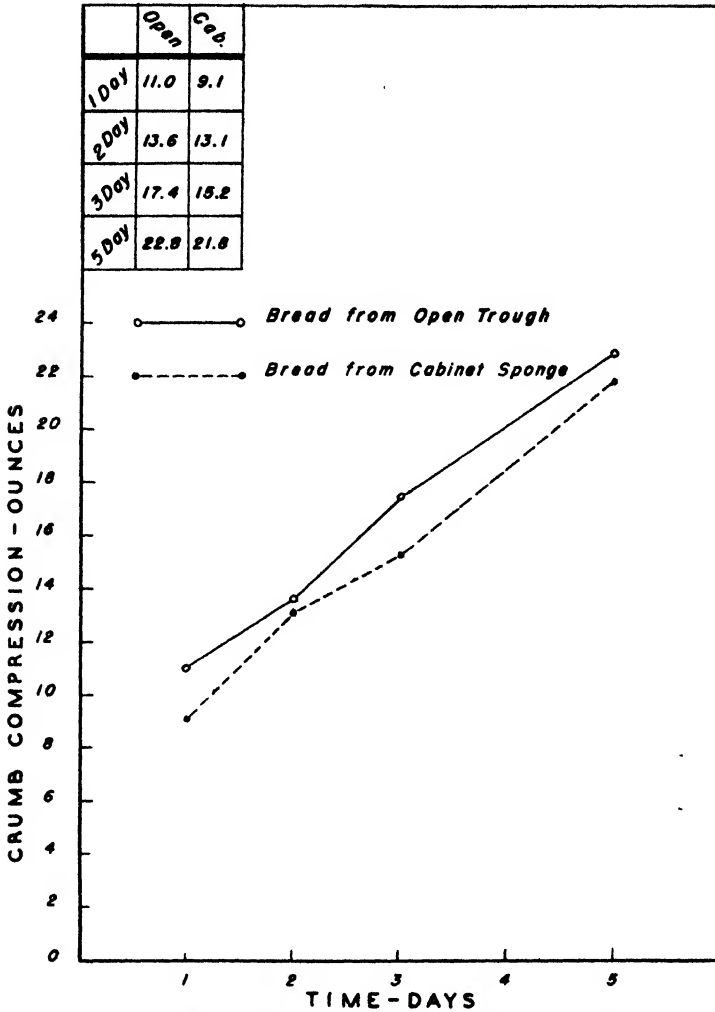


Fig. 23. Crumb compression measurements on bread from comparable open-trough and cabinet sponges made after 1, 2, 3, and 5 days holding at room temperature. Test 3, utilizing bread made from hard winter wheat flour (1944 crop).

results obtained with the bread made from hard winter wheat flour. The bread resulting from the cabinet sponge appears to be slightly softer than that made from the open-trough sponge, but in the light of the authors' experience in the application of this test, the difference is just barely significant.

Under the conditions of this study, therefore, the cabinet is ineffective except to insulate the sponges against significant fluctuations in dough-room temperature. It usually maintains a slightly higher

temperature than the dough room, and automatically provides sufficient humidity to prevent crusting of the sponges.

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MALTASE ACTIVITY OF VARIOUS MOLD BRANS AS EVALUATED BY SELECTIVE FERMENTATION¹

ROBERT L. GATES² and ERIC KNEEN³

ABSTRACT

A simplified, roughly quantitative procedure for the estimation of maltase activity was developed. The method involves the digestion of maltose by enzyme extracts under specified conditions and the evaluation of glucose produced by a differential fermentation procedure. A yeast having a very low maltose fermentation capacity in the first stages of fermentation was used, and carbon dioxide production in the first hour following an initial 30 minutes of fermentation was measured manometrically and related to glucose content.

When applied to the study of mold brans prepared by culturing various fungal isolates, the method indicated a wide range in maltase activities. Some mold brans of low amylase activity showed high maltase activity, while in others, high amylase activity was no guarantee of high maltase activity. However, within a group of closely related isolates, for example, the *Rhizopus-Mucor* or the *Aspergillus oryzae*, there was a positive correlation between amylase and maltase activities.

There is considerable evidence to support the contention that the principal product from the action of the amylases on starch is maltose (5, 6, 14, 16). Indicative of the general acceptance of this fact is the custom of reporting amylase activity in terms of grams of maltose produced. To hydrolyze maltose to the fundamental unit of starch structure, glucose, a third enzyme must be brought into play. This enzyme, maltase or alpha-glucosidase, is frequently absent from amylase-active materials.

The exact role or importance to be ascribed to maltase in the various processes that involve the degradation of starch is open to question. Leibowitz and Hestrin (8) have furnished an excellent review of the possible role of maltase in the fermentation of maltose. They insist that the enzyme is unnecessary for the action of bakers' yeast on maltose but concede that it may play a part in fermentation of maltose by brewers' yeast. Contradictory evidence of the superficiality of maltase in the fermentation picture has been submitted by Corman and Langlykke (3). These workers showed that the alcohol production potential of a fungal saccharifying agent for corn mashes

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was more closely correlated with a "gluconic enzyme system, as measured by maltose hydrolysis, than with fungal alpha-amylase."

Certainly the action of maltase is important to those interested in the production of a syrup with a controlled glucose/maltose ratio by the enzymic degradation of starchy materials. Schultz *et al.* (12) discuss the matter of glucose and maltose content of syrups and compare the results of analysis by chemical and selective fermentation methods. Schwimmer (14) suggested that maltase has an important role in the degradation of raw starches by the removal of the end products of amylase action and elimination of certain inhibitory effects. From a theoretical point of view the maltase activity of an enzyme-containing material should be measured in order to complete the characterization of the carbohydrase system.

The measurement of maltase activity can be a tedious procedure due to the difficulties involved in the determination of glucose in the presence of maltose (9, 10). Many have turned to the selective fermentation method of Somogyi (15) who found that alkaline conditions suppressed the fermentation of maltose but not that of glucose. A similar method has been developed by Schultz and Kirby (13) in which they employ certain specific organisms for the removal of individual sugars from mixtures of several carbohydrates. Several workers have reported the differences that exist in the rate of fermentation of glucose and maltose by a given yeast (2, 4). Maltose requires a definite induction period before active fermentation occurs.

It seemed possible that the principle of selective fermentation might be applied by utilizing the unusual properties of one of the commercial dehydrated yeasts. The purpose of this report is to present the development of a method of maltase assay using a commercial dehydrated yeast and the application of the method to the screening of some 43 carbohydrase systems.

Methods

Extraction of Mold Bran. The mold brans were extracted for 1 hour at 30°C. with distilled water in a ratio of 10 g. of bran to 100 ml. of water. The extracts were then filtered through paper and desired aliquots used.

Fermentation Measurement. The carbon dioxide produced by yeast fermentation was evaluated manometrically in the "pressure-meter" described by Sandstedt and Blish (11) using mercury manometers. The aluminum cups proved very adaptable because they provide a rapid heat transfer. The method was similar to that applied to the evaluation of amylase "saccharifying activity" by Kneen and

Beckord (7), whose suggested yeast nutrient, a modification of the Atkin, Schultz, and Frey (1) nutrient, was used.

Results and Discussion

Selective Fermentation by Commercial Yeasts. Three commercial yeasts were tested for fermentation of maltose and glucose. One of these, "A," was a compressed product and the other two, "B" and "C,"⁴ were dehydrated products. For the fermentations each pressuremeter cup contained, in addition to the nutrients, 600 mg. sugar and 0.5 g. yeast, the total liquid volume being 30 ml. In Table I are recorded in abbreviated form the data for the first part of the fermentations.

TABLE I
SELECTIVE FERMENTATION OF MALTOSE AND GLUCOSE BY THREE
COMMERCIAL YEASTS
(Carbon dioxide production expressed as mercury pressure)

Yeast ¹	Glucose (600 mg.)			Maltose (540 mg.), glucose (60 mg.)			Maltose (600 mg.)			
	1 hr.	2 hr.	5 hr.	1 hr.	2 hr.	5 hr.	1 hr.	2 hr.	5 hr.	8 hr.
"A"	mm. 90	mm. 254	mm. 414	mm. 70	mm. 216	mm. 484	mm. 17	mm. 81	mm. 382	mm. 437
"B"	147	307	455	28	73	424	12	21	185	411
"C"	119	277	454	23	42	153	10	14	30	57

¹ "A," compressed; "B" and "C," dehydrated.

It is obvious from Table I that the customary "induction period" with maltose was even more pronounced for the two dehydrated yeasts used than for the compressed yeast. This was particularly true of the "Maca" yeast. The present as well as previous studies encouraged the belief that a simple, rapid, roughly quantitative evaluation of glucose in the presence of maltose could be achieved by the use of this particular yeast.

Considerable experimentation made it evident that if 1.5 g. of "Maca" yeast was used per pressuremeter cup and the 15-minute manometer reading subtracted from the 75-minute reading, the difference was roughly proportional to glucose content and not influenced by maltose concentration. This is shown by the data of Table II.

Further investigations were made to establish the most nearly quantitative conditions for the fermentation. A range of yeast con-

⁴ The "C" dehydrated yeast was marketed as "Maca." Subsequent to the completion of much of the research we were informed by the Northwestern Yeast Company that this product has been withdrawn from the market. Without doubt other nonmaltose fermenting yeast strains would serve as efficiently.

centrations from 0.5 to 2.0 g. per cup was used and the carbon dioxide production between 15 and 75 minutes, between 30 and 90 minutes, between 45 and 105 minutes, and between 1 and 2 hours of fermentation was correlated with actual glucose present. This information supported the previous evidence that 1.5 g. yeast was most satisfactory.

The 1-hour period during the course of the fermentation in which carbon dioxide production was most closely related to glucose content was finally determined to be that between 30 and 90 minutes rather than between 15 and 75 minutes of fermentation. These data are

TABLE II
FERMENTATION OF GLUCOSE BY "MACA" YEAST IN THE ABSENCE
AND PRESENCE OF MALTOSE

Sugar present -- glucose		Carbon dioxide production 75-15 min.	Glucose calculated ¹
mg.		mm.Hg.	mg.
600		317	600
540		294	556
480		252	478
420		220	417
360		180	341
240		113	214
120		59	112
60		31	59

Sugar present		Carbon dioxide production 75-15 min.	Glucose calculated ¹
Glucose	Maltose		
mg.	mg.	mm.Hg.	mg.
600	—	301	600
540	60	280	558
480	120	255	509
420	180	214	427
360	240	178	335
240	360	119	237
120	480	62	124
60	540	31	62

¹ Based on the assumption that glucose only is fermented and that a proportional relationship exists between mm.Hg. pressure and glucose present.

given in Table III. In every set of determinations the corresponding fermentation value for 600 mg. glucose was determined. The "calculated" quantity of glucose for any one manometer reading was derived by assuming an exactly proportional relationship between carbon dioxide produced and glucose present.

The data in Table III indicate that carbon dioxide production in the 1-hour interval following the first 30 minutes of fermentation is most closely related to glucose quantity over a range of sugar concentrations. The definite limitations in replicability of the procedure

likewise are evident. At best the method can be considered as only roughly quantitative. Also shown in Table III is further evidence that the presence or absence of maltose has no influence on the glucose calculation.

Determination of Maltase Activity. With a procedure available for estimating glucose in the presence of maltose it became possible to evaluate maltase activity. To bear some relationship to certain industrial applications it was desired to determine the extent of such activity occurring during a 1-hour period at temperatures in the neighborhood of 50°C. The procedure developed is as follows:

Three aluminum pressuremeter cups are used for each determination. Each contains 0.06 g. sodium chloride, 0.04 g. crystalline

TABLE III
RELATIONSHIP OF CARBON DIOXIDE PRODUCTION TO GLUCOSE QUANTITY
DURING THE ONE-HOUR INTERVAL FOLLOWING THE FIRST
15, 30, 45, AND 60 MINUTES OF FERMENTATION
(Quantity of "Maca" yeast—1.5 g.)

Sugar present		Calculated glucose from pressuremeter readings for 1-hour intervals			
Glucose	Maltose	15 and 75 min.	30 and 90 min.	45 and 105 min.	60 and 120 min.
mg.	mg.	mg.	mg.	mg.	mg.
120	—	168	132	108	—
240	—	306	276	240	228
240	—	234	240	246	246
240	360	276	240	228	240
360	—	408	360	336	342
480	—	504	486	450	450
480	—	504	480	486	510
480	120	522	482	456	462

magnesium sulfate, 0.04 g. potassium dihydrogen phosphate, 0.011 g. potassium monohydrogen phosphate, 0.0008 g. thiamine hydrochloride, and 0.0008 g. pyridoxine in 15 ml. of solution (pH 5.8). To the first cup is added 600 mg. glucose and to each of the second and third cups 600 mg. maltose. Cups one and two are placed in a 50°C. water bath and brought to temperature (3 minutes), and the "blank" cup, No. 3, is placed in a boiling water bath. At appropriate intervals, aliquots of the enzyme solution to be evaluated are added to the three cups either in 10 ml. volume or accompanied by sufficient water to give a total cup volume of 25 ml. The enzyme aliquots should be such that 40% conversion of the maltose is not exceeded during the reaction, for reasons to be discussed later. Each cup is rotated rapidly upon addi-

tion of the enzyme to mix and to bring the contents rapidly to the desired temperature. Each cup is covered with a watch glass to minimize evaporation. After 3 minutes in the boiling bath the "blank" cup containing nutrient, maltose, and inactivated enzyme may be cooled and carried along with the enzyme tests.

After 1 hour at 50°C. each cup is placed in a boiling water bath for 3 minutes and is then cooled in running cold water. On a new selected time schedule the cups are brought to 30°C., 10 ml. of yeast suspension containing 1.5 g. of "Maca" yeast added to each, and the manometers affixed. The pressure is read in mm. mercury after 30 minutes of fermentation and again after 90 minutes. The carbon dioxide produced in the indicated time interval with the blank is subtracted from the interval readings found for the two cups containing active enzyme. The glucose produced in the cup is calculated as follows:

$$\frac{\text{Corrected maltose reading}}{\text{Corrected glucose reading}} \times 600 \text{ mg.} = \text{mg. glucose produced}$$

No detailed studies of the influence of time, temperature, or pH on the activity of any one fungal enzyme were made. The object of the investigation primarily was to evaluate the maltase activity of a wide variety of products under more or less rigidly stipulated conditions. The optimum activity conditions for one product might bear little relationship to those for another. The conditions used, 1 hour at 50°C. and pH 5.8, are known to be favorable if not optimum for the maltase activity of at least several fungal products.

Calculation of Maltase Activity. The data obtained by the procedure represent the glucose produced from 600 mg. maltose by an extract aliquot equivalent to a given weight of mold bran. For comparison of various materials it is requisite that this be proportional to maltase quantity.

In Fig. 1 is shown the relationship between the quantity of mold bran and the amount of maltose converted in 1 hour at 50°C. Two different molds are represented, a strain of *Aspergillus oryzae* and one of *A. niger*. The bran produced by culturing *A. oryzae* had about twice the activity of the *A. niger* bran.

It is apparent that maltose conversion is directly proportional to mold bran quantity only at low conversion values. Activity evaluations at one mold bran concentration may be compared by calculating the ideal conversions that would result from the first small increments of bran. If the two curves have the same characteristics over an appreciable range, one curve may be drawn relating the actual maltose

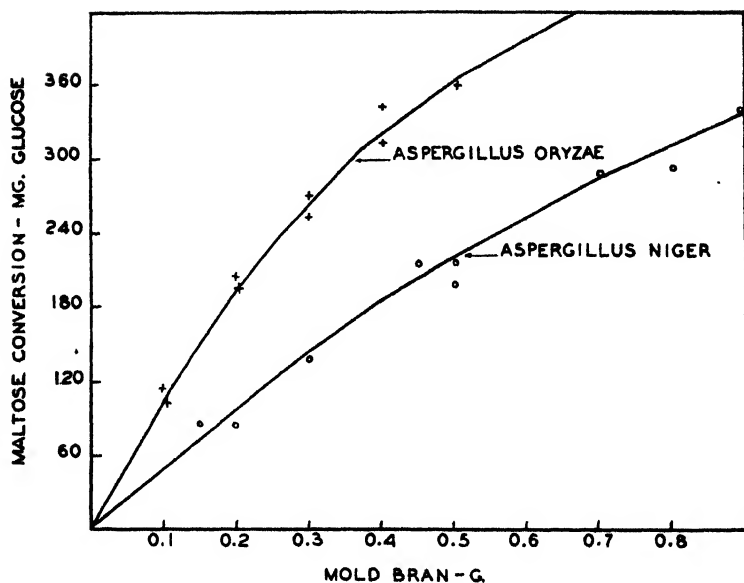


Fig. 1. Relation between maltose conversion and quantity of two mold brans.

converted to the conversion that would have resulted if the initial enzyme-maltose conversion relationship had persisted in an ideal linear fashion. For each of the mold brans the "found" values at any one concentration (taken from the curves of Fig. 1) were compared with the values that would have resulted if the initial bran-quantity

TABLE IV
RELATIONS BETWEEN "FOUND" AND "IDEAL" MALTASE CONVERSION
BY INCREASING QUANTITIES OF TWO MOLD BRANS
(Ideal conversions calculated by linear extrapolation
of initial low-quantity conversions)

Wt. mold bran <i>A. oryzae</i>	Glucose found	Glucose ideal	Wt. mold bran <i>A. niger</i>	Glucose found	Glucose ideal
g.	mg.	mg.	g.	mg.	mg.
0.05	54	54	0.05	24	24
0.1	105	108	0.1	48	48
0.15	150	162	0.2	90	96
0.2	195	216	0.3	132	144
0.25	234	270	0.35	152	168
0.3	264	324	0.4	174	192
0.35	297	378	0.5	210	240
0.4	324	432	0.6	246	288
0.5	369	540	0.7	279	336
			0.8	312	384
			0.9	342	432
			1.0	369	480

maltose-conversion relationships had persisted in linear proportionality throughout higher concentrations. The results are given in Table IV and are shown diagrammatically in Fig. 2. The two curves essentially coincide up to about 40% conversion of maltose (240 mg. glucose produced). Accordingly, below this limiting value a "found" conversion value for mold bran may be "corrected" to a figure linearly proportional to enzyme quantity by referring to the curve. By using the calculated figure, a unit value for a mold bran may be determined that can be related to a similarly calculated value for another product. The arbitrary *maltase unit value* selected for the work is the *milligrams*

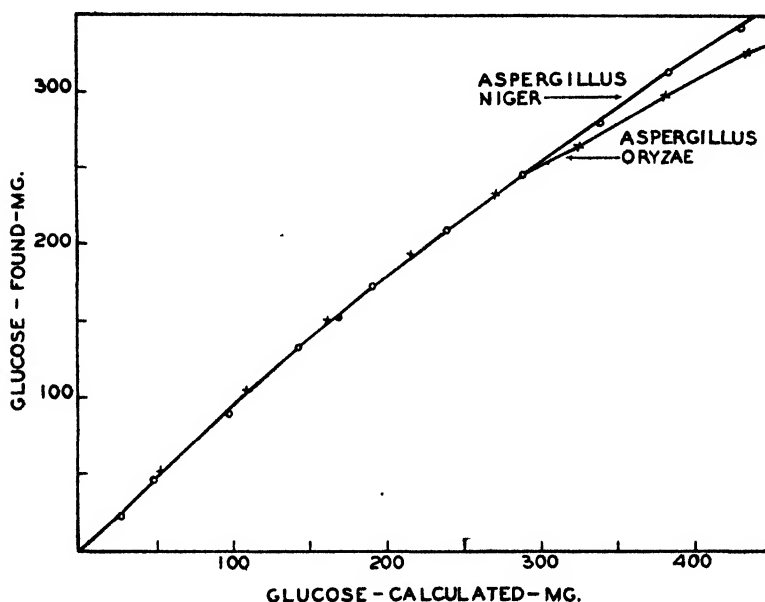


Fig. 2. Relation between "found" and "ideal" maltose conversions for increasing quantities of two mold brans. The "ideal" conversions are calculated by linear extrapolation of the relations found for initial small increments as shown in Fig. 1.

of maltose converted to glucose by the action of 1 g. of mold bran in 1 hour at 50°C. Observance of the reaction conditions is assumed, i.e., 600 mg. maltose as a substrate, pH 5.8, 25 ml. liquid volume, and a conversion limit of 40%.

The glucose present in the cup after the reaction is calculated from the fermentation data. By the use of the relationship shown in Fig. 2 the calculated amount of glucose representing linear proportionality between enzyme concentration and glucose production is determined. Based on the weight of dry enzymic material used for maltose conversion, the unit value can be obtained. For example, if an extract

aliquot equivalent to 0.5 g. mold bran (10% moisture) is used for the reaction and 200 mg. glucose is produced (corrected to 225 mg. by Fig. 2) the maltase activity of the material would be $\frac{225}{0.9 \times 0.5}$ or 500 units.

Maltase Activity of Mold Brans. The maltase evaluation procedure and method of calculation was applied to 41 experimental brans prepared by culturing various fungal isolates on wheat bran medium. Those brans along with their amylase activities were kindly supplied by Dr. George L. Peltier of the University of Nebraska. A description of the mold brans together with their amylase and maltase activities is given in Table V. Also included for comparison are the results for

TABLE V
AMYLASE AND MALTASE ACTIVITY OF VARIOUS MOLD BRANS

Fungal isolate ¹	Organism	Amylase activity ²	Maltase activity
<i>Aspergillus</i> type			
		<i>min.</i>	<i>units</i>
Commercial "A"	<i>oryzae</i>	1.0	850
FA 35	<i>oryzae</i>	2.3	297
FA 38	<i>oryzae</i>	2.9	265
FA 38	<i>oryzae</i>	3.4	255
Commercial "B"	<i>oryzae</i>	13.0	164
FA 36	<i>oryzae</i>	3.6	145
MF 309	black	180	415
MF 349-430	black	180	407
FA 10C	green	3.0	297
MF 19	green	19.0	255
MF 375	green	28	92
MF 347	yellow-gold	54	73
MF 417	yellow-gold	70	48
MF 30	tan	120	42
MF 321	tan	230	42
MF 155	clavate heads	1430	73
MF 85	clavate heads	1060	60
MF 377	miscellaneous	920	66
MF 413	miscellaneous	1020	60
MF 39	miscellaneous	72	48
MF 367	miscellaneous	70	36
MF 473	<i>Rhizopus-Mucor</i>	60	297
MF 469	<i>Rhizopus-Mucor</i>	75	205
MF 467	<i>Rhizopus-Mucor</i>	68	177
MF 426	<i>Rhizopus-Mucor</i>	83	140
MF 43	<i>Rhizopus-Mucor</i>	200	98
MF 672	<i>Rhizopus-Mucor</i>	218	66

TABLE V—*Continued*

Fungal isolate ¹	Organism	Amylase activity ²	Maltase activity
<i>Penicillium</i> type			
		<i>min.</i>	<i>units</i>
FA 1C	<i>Penicillium</i>	80	280
WF 265	blue-green	76	140
MF 49	blue-green	160	30
MF 275	gray-green	2000	135
MF 460	gray-green	880	98
MF 541	gray-green	990	66
MF 257	gray-green	730	30
F 14C	yellow-green	730	60
MF 509	<i>Alternaria</i> -like	160	98
MF 47	<i>Alternaria</i> -like	135	36
MF 202	mixed culture	660	36
MF 157	mixed culture	96	36
MF 255	unclassified	290	36
MF 7	unclassified	43	24
MF 76	unclassified	100	24
MF 495	<i>Trichoderma</i>	1640	12

¹ With the exception of the two commercial mold brans the designations are those supplied by Dr. George L. Peltier of the University of Nebraska and to be presented by him in a publication now in press.

² Amylase activity—dextrinizing time for 0.05 g. mold bran; amylase activity is inversely proportional to the values recorded.

two commercial mold brans. The amylase activities are not calculated as "unit" values but are given merely as "dextrinization time for 0.05 g. bran" (i.e., the time required for 0.05 g. mold bran to dextrinize 20 ml. of a 1% starch suspension at pH 5.0 and 30°C.). Accordingly, amylase activity is inversely proportional to the value given.

It is apparent that there is very little correlation between amylase activity and maltase activity, with the exception that the *Aspergillus oryzae* brans tended to be high in both activities. However, two mold brans cultured with *A. niger* isolates were exceeded in maltase activity only by one of the "commercial" products, notwithstanding that their amylase activities were exceeded by 26 of the 43 brans evaluated. There is an interesting relation between the activities of the two enzymes within a group classification. For example, in the *A. oryzae* and the *Rhizopus-Mucor* groups the order of activity is about the same for each enzyme, indicating a positive correlation between the amylase and maltase production capacities of a given organism.

Application of the Procedure. One application of a method for maltase activity has been illustrated above—the testing of numerous mold brans prepared by culturing isolates with unknown potentialities. Most of the other investigations were not carried out under exactly the same conditions described here so they will not be outlined in detail. However, of the various materials tested, barley malt proved to have no measurable maltase activity. Of three “bacterial brans” tested, one commercial and two supplied by Dr. George L. Peltier, the commercial had no maltase activity and the two Nebraska isolates (W-96 and P-65) had about half the activity of those commercial mold brans available on the market. The several commercial mold brans tested, from various sources, had similar intermediate maltase activities comparable in magnitude to the “Commercial B” sample of Table V. The “Commercial A” mold bran of Table V was a special, very active product not available in large quantity.

The maltase procedure likewise proved of value in studies on the concentration of the mold bran carbohydrase system. A mold bran extract and dispersions of isopropyl and ethyl alcohol precipitates obtained from it were compared at equal amylase activities. The isopropyl alcohol precipitate had 76% as much maltase activity as the extract of original bran, whereas the ethyl alcohol precipitate had only 30% of the original maltase activity. This demonstrated that, at levels of alcohol satisfactory for amylase precipitation, isopropyl alcohol was more efficient than ethyl alcohol in the precipitation of maltase and its recovery in an active form.

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INFLUENCE OF SALTS ON AMYLASE ACTIVITIES OF MALT¹

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ABSTRACT

Salts added in small amounts to distilled water in the extraction of barley and wheat malts increase the alpha-amylase activities in the extract strongly but variably. At salt concentrations of approximately 0.1 *N* or less, respective optimum activities are reached, whereas further salt additions may depress the activity more or less, depending upon the specific ions present. Judicious selection of salts yielded highest optimum or "maximum" alpha values which are not increased by raising the temperature of extraction from 20° and 30° to 40°C., or by increasing the duration of extraction. These "maximum" alpha activities appeared to be characteristic of the respective malt, and close to those obtainable under industrial mashing conditions. The ratio of these "maximum" alpha activities to those determined in distilled water extracts varies considerably from one malt to the other.

Extraction of barley malt with dilute salt solutions had little or no effect on beta-amylase activity, whereas two wheat malts contained large quantities of salt-soluble beta-amylase. Higher salt concentrations may depress beta-amylase activity depending upon the specific ions, but to different extents than alpha-amylase.

The influence of the salts added before extraction was compared with that after extraction. At low salt concentrations no activation was found for alpha-amylase after extraction, while slight stimulations of beta-amylase occurred with some ions. The addition of high salt concentrations to the extracts decreased the activity of both alpha- and beta-amylase. The lower amylase values obtained when malts are extracted with salts of high concentrations are due to the inhibitory effects of the ions on enzyme activity rather than to lower solubility of these enzymes in such salt solutions.

The effect of papain upon the release of alpha-amylase appeared to be significantly different from that of latent beta-amylase.

The influence of proteolytic enzymes and salts on the extraction or release of the amylases from wheat and barley and from their malts has

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long been known. It has been observed also that the activities of aqueous solutions of alpha- and beta-amylase are affected by the presence of electrolytes, especially of metal salts, the effect depending upon the nature of the ions, the concentrations, and the pH of the solution (1, 2, 12, 28, 29, 34-39). The alpha-amylases of both cereals and noncereals are partially protected against inactivation by heat, high acidity, and inhibitors by the presence of small amounts of calcium salts (8, 9, 16, 18, 20, 21, 23, 30, 40, 42). It has been reported that the liquefaction of grain mashes with malt is improved when calcium sulfate is added (41), and additions of calcium sulfate or calcium chloride and of sodium chloride have been recommended in the analytical determination of malt alpha-amylases to obtain higher and more reproducible results (13, 17, 19, 21, 43).

However, the precise effect of salts on the extraction of the amylases from malt has not been clearly established. Since inorganic salts in varying low concentrations are always present in the mash waters in the commercial conversion of starch, a study was undertaken to determine the effect of different salts, particularly of those found in industrial waters, upon the extraction of amylases from malt and also upon the activity of extracts made with distilled water.

Materials and Methods

The majority of the experiments were conducted with commercial brewers' and distillers' malts; the former are dried at final temperatures of 70° to 75°C. and the latter at about 50°C. In addition, green barley malts, dried in vacuo in the laboratory, and two wheat malts were examined. Each sample was thoroughly mixed and stored in airtight containers.

In principle, the methods used for extracting the malt, conducting the ensuing starch hydrolysis, and determining diastatic power followed those outlined in *Cereal Laboratory Methods* (4th ed. 1941), while dextrinizing activity was determined by the method of Sandstedt, Kneen, and Blish (32) as modified by Olson, Evans, and Dickson (25, 26) and by Redfern (31).

Ten grams of the finely ground malt were extracted with 200 ml. of distilled water or the particular salt solution under study at temperatures of 20°, 30°, and 40°C. for times varying from 10 minutes to 22 hours. Blank tests were made at least daily. The alpha-amylase activity was calculated in terms of Sandstedt, Kneen, and Blish units at 20°C. ("20° dextrinizing units") (26). The beta-amylase activity was calculated as the difference between the maltose equivalents of the

total saccharifying activity or diastatic power and that of the alpha dextrinizing activity. The equivalents given by Olson, Evans, and Dickson (25) were employed. They are in good agreement with those of other workers (13), between about 15 and 35 "20° dextrinizing units," but differ somewhat at lower and higher activities. The reliability of these beta-amylase values is naturally influenced by errors in both measurements from which they are calculated.

Results

Variations in Amylase Activities of Distilled Water Extracts of Brewers' Malt upon Storage. Subsamples from a uniform lot of one brewers' malt ("A") of medium kernel size were extracted with distilled water at 20° and 30°C. respectively after various intervals of storage up to 115 days and the alpha-amylase, diastatic power, and beta-amylase of the extracts determined at 20°C. The results given in Table I show that the alpha-amylase values vary considerably more than those for diastatic power, and consequently also more than beta-

TABLE I
AMYLASE ACTIVITIES DETERMINED ON ONE BREWERS' MALT
AFTER VARIOUS INTERVALS OF STORAGE¹

Age of sample	Extraction at 20°C.			Extraction at 30°C.		
	Alpha S.K.B. units	D.P.	Beta maltose equiv.	Alpha S.K.B. units	D.P.	Beta maltose equiv.
<i>Days</i>		<i>°L.</i>			<i>°L.</i>	
2	17.8	95	320	—	—	—
6	17.8	96	324	—	—	—
43	14.7	95	332	—	—	—
44	14.7	98	344	19.4	101	340
51	14.7	99	348	—	—	—
57	15.6	95	328	—	—	—
59	16.7	97	332	—	—	—
64	16.7	94	320	—	—	—
66	15.6	94	324	19.4	95	316
73	15.6	94	324	19.4	95	316
77	14.7	95	332	—	—	—
79	16.7	96	328	—	—	—
81	16.7	96	328	20.0	98	324
82	15.6	94	324	—	—	—
84	16.7	96	328	—	—	—
97	14.7	97	340	—	—	—
107	17.8	95	320	22.7	97	312
115	16.6	96	328	—	—	—
Mean	16.1	96	328	20.6	97	320
Standard deviation	1.12	1.41	8.0	1.43	2.5	11.4
Coefficient of variability, %	7.0	1.5	2.4	6.9	2.6	3.6

¹ Extraction with distilled water for 2 hours; hydrolysis at 20°C.

amylase. The variations in alpha-amylase are apparently not correlated with the duration of storage. The pH of the extracts varied from 5.5 to 5.8, but there was no apparent relation between these values and those for alpha-amylase activity.

The extracts made at 30°C. gave higher alpha-amylase activities than those prepared at 20°C., but increasing the extraction temperature had no marked influence on the beta-amylase values.

Influence of Various Salts on the Extraction of Amylases from Barley Malt. Several commercial barley malts, brewers' and distillers', were extracted with distilled water and with 0.001 to 2.0 *N* solutions of different salts and other agents, such as citric acid and calcium hydroxide, for 2 hours at 20° and at 30°C. The amylase activities were

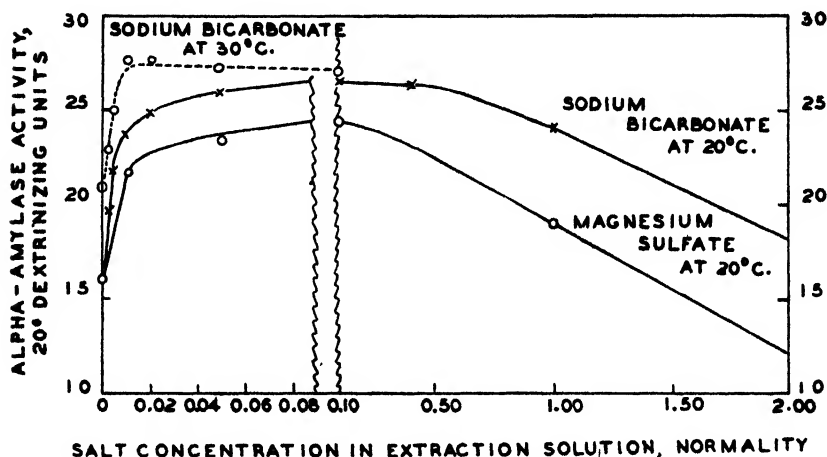


Fig. 1. Effect of salt additions on the extraction of alpha-amylase of one brewers' malt, at 20° and 30°C. for two hours

determined at 20°C. for both temperatures of extraction. The concentrations were compared on normality basis whereby—in deviation from usual practice—the hydrogen ion of acid salts was considered equivalent to a metal ion; consequently, bicarbonates and primary or secondary phosphates were calculated as di- and tri-valent respectively.

The smallest salt additions influenced the diastatic power, but much more the alpha-amylase activities.

The effect on alpha-amylase activity showed the same pattern for all the malts tested and all the salts used. These activities are sharply increased in the lowest bracket of salt concentrations. They reach optimum values below or at about 0.1 *N*, and decline more or less at higher concentrations. This is illustrated in Fig. 1 by typical curves

which show the effects of increasing salt concentrations of sodium bicarbonate and magnesium sulfate on alpha-amylase activity.

Other salts tested on the same malt ("A") yielded optimum levels of alpha-amylase which varied between those of the two examples, but none gave a higher optimum activity than sodium bicarbonate.

To provide a common basis for comparing the results with different malts, the alpha-amylase activities were expressed in per cent of the "maximum" value, that is the value obtained for each malt by extraction with 0.1 *N* sodium bicarbonate at 30°C. for 2 hours. The diastatic power and beta-amylase values were expressed in per cent of the corresponding activities for the extracts made with distilled water at 20°C. The results, when calculated on this basis, showed a marked similarity for the different barley malts extracted with the same salt solutions. For the brewers' malts investigated in this study, these per cent values were found to be practically identical, except at concentrations below about 0.01 *N* (especially in the most dilute solutions).

Representative results obtained with three brewers' malts and several selected concentrations of the various substances are given in Table II. These brewers' malts had the following characteristics:

<i>Origin</i>	<i>Diastatic power °L.</i>	<i>"Maximum" alpha-amylase activity units</i>
"A," South Dakota	96	28
"B," North Dakota	127	33
"C," Minnesota	137	40

The alpha-amylase activities were increased to within about 5% of the "maximum" value by appropriate concentrations of the first eight salts; the other substances gave increases which were 10% or more below the "maximum." In all cases the lowest salt concentrations gave higher values when the extractions were conducted at 30° than at 20°C. As the concentrations were increased, the differential effect of extraction temperature became less pronounced and practically disappeared at about 0.1 *N*, the concentration at which the respective highest activities were reached.

Relatively insignificant increases in beta-amylase activity occurred with any of the salts, in contrast to their very marked effects on alpha-amylase. Some salts such as the alkali chlorides and sulfates and magnesium sulfate were without appreciable effect on beta-amylase activity in concentrations up to 2.0 *N*, whereas others such as the nitrates, acetates, and bicarbonates of sodium and potassium, and more particularly calcium and magnesium chloride and sodium sulfo-

TABLE II
EFFECT OF EXTRACTING BREWERS' MALT WITH VARIOUS
SOLUTIONS ON AMYLASE ACTIVITIES¹

Extraction medium	Salt concentration	Alpha-amylase extracted at		Diastatic power extracted at		Beta-amylase extracted at		pH of extract
		20°	30°	20°	30°	20°	30°	
Distilled water	N —	% 58	% 74	% 100	% 102	% 100	% 98	5.5–5.8
Sodium bicarbonate	0.002	69	81	105	110	104	105	6.2
	0.005	81	89	110	110	106	103	6.6
	0.01	85	100	110	113	103	103	7.0
	0.05	89	96	106	116	99	107	7.7
	0.10	94	94	109	117	100	107	7.9
	1.00	85	89	98	97	88	87	8.2
	2.00	64	56	95	98	91	94	8.3
Potassium bicarbonate	0.01	84	94	—	113	—	103	6.2
	0.05	87	94	107	117	98	108	6.9
	0.10	90	94	—	117	—	108	7.3
	0.50	87	90	104	112	96	104	8.0
	2.00	—	83	—	97	—	88	8.2
Sodium nitrate	0.01	76	80	106	107	100	101	5.8
	0.05	91	92	106	107	99	100	5.8
	0.10	100	92	106	107	96	99	5.8
	1.00	91	96	104	105	95	92	5.8
	2.00	89	89	105	100	95	90	5.8
Sodium acetate	0.01	75	83	114	—	106	—	5.7
	0.10	92	92	106	108	104	107	6.1
	0.50	100	89	—	—	—	—	6.7
	2.00	53	58	71	75	72	75	7.2
Disodium hydrogen phosphate	0.01	89	89	110	110	105	105	6.8
	0.10	92	92	112	112	107	107	7.3
	0.50	89	89	112	112	108	108	7.8
Diammonium hydrogen phosphate	0.01	89	92	103	108	97	103	6.3
	0.10	92	100	107	110	101	103	7.3
	0.50	92	92	105	106	99	102	7.4
	2.00	75	75	76	82	68	76	7.9
Sodium sulfocyanate	0.01	81	85	105	108	96	97	5.8
	0.05	95	95	104	106	93	96	5.75
	0.10	95	95	105	108	96	97	5.7
	1.00	81	49	59	38	45	28	5.7
Sodium citrate	0.01	83	—	107	—	102	—	6.2
	0.05	94	—	107	—	98	—	6.6
	0.10	91	—	106	—	98	—	6.7
	1.00	65	—	93	—	89	—	7.1

¹ Extraction for 2 hours at 20° and at 30°C. Amylase determinations in extracts at 20°C. Alpha-amylase calculated in per cent of respective "maximum" value, diastatic power and beta-amylase in per cent of "distilled water" values.

TABLE II—*Continued*

Extraction medium	Salt concentration	Alpha-amylase extracted at		Diastatic power extracted at		Beta-amylase extracted at		pH of extract
		20°	30°	20°	30°	20°	30°	
	<i>N</i>	%	%	%	%	%	%	
Chlorides and sulfates of sodium, potassium, and ammonium	0.002	63	63	101	101	100	100	5.7
	0.005	73	73	104	104	100	100	5.7
	0.01	78	78	105	107	100	102	5.7
	0.05	84	84	109	109	101	101	5.7
	0.10	84	84	110	108	102	100	5.7
	1.00	90	85	107	106	100	100	5.6
Potassium chloride	2.00	85	85	108	114	100	106	5.4
Sodium chloride	2.00	71	69	—	103	—	99	5.3
Potassium sulfate	2.00	64	59	95	99	93	99	5.3
Ammonium sulfate	2.00	47	53	94	90	90	96	5.4
Calcium acetate	0.01	71	76	103	105	100	102	6.8
	0.10	84	84	108	109	101	103	6.5
	1.00	77	80	97	98	95	91	6.7
	2.00	71	74	97	96	91	89	6.9
Calcium chloride	0.01	72	79	103	106	100	102	5.4
	0.10	79	83	103	107	99	103	5.6
	0.50	77	81	—	—	—	—	6.1
	1.00	77	80	102	101	96	93	6.3
	2.00	53	53	24	11	14	0	7.0
Magnesium chloride	0.01	75	92	—	—	—	—	5.5
	0.10	79	89	111	112	108	108	5.4
	0.50	83	89	—	—	—	—	4.8
	2.00	50	21	72	46	71	49	4.6
Magnesium sulfate	0.01	78	85	102	110	97	100	5.5
	0.05	84	87	105	108	96	102	5.4
	0.10	89	91	100	108	94	101	5.3
	1.00	67	62	98	96	95	94	5.3
	2.00	44	47	88	87	90	88	5.6
Potassium dihydrogen phosphate	0.01	68	75	99	102	98	103	5.6
	0.10	75	78	105	101	103	100	5.3
	0.50	75	75	103	101	100	99	4.9
	2.00	33	0	97	86	105	101	4.4
Citric acid	0.005	31	—	85	—	94	—	4.6
	0.01	0	—	52	—	61	—	4.3
	0.05	0	—	4	—	4	—	3.1
Calcium hydroxide	0.02	89	89	86	60	73	42	8.5
	0.04	89	89	19	19	0	0	9.4
	0.10	74	74	17	15	0	0	11.2
Calcium peroxide	0.01	81	—	91	—	81	—	6.4
	0.05	81	—	17	—	0	—	8.6
Ammonium persulfate	0.01	56	—	91	—	88	—	5.2
	0.05	18	—	67	—	73	—	4.8
Potassium periodate	0.01	25	—	6	—	0	—	4.9
	0.05	0	—	0	—	0	—	4.15

cyanate, reduced the activity at the higher concentrations, especially when the extractions were made at 30°C.

The increase in diastatic power, which represents the combined saccharifying activities of both amylases, at low salt concentrations is due, primarily or exclusively, to the increase in alpha-amylase. At the higher salt concentrations, the decrease in diastatic power is influenced by the depressing action of the salts on either amylase or on both.

Effect of Various Salts on the Activity of Malt Amylases in Solution. Samples of a brewers' malt ("A") were extracted with distilled water at 20° and 30°C. for 2 hours and the solutions filtered. Various salts were then added to give the same series of concentrations as were used in the extraction experiments outlined in the previous section. The amylase activities were determined at 20°C. and the results expressed in per cent of the "distilled water" values without any salt addition. These per cent values were compared with the corresponding extraction figures in which the alpha-amylase activity was again expressed in per cent of the "maximum" activity and the beta-amylase in per cent of the "distilled water" extraction value at 20°C. The results are recorded in Table III.

For alpha-amylase no marked activation occurred when the salts were added after extraction; thus the strong increases of the extraction values could not be due to an activating influence of the salts upon the enzyme. Some salts depressed the activity of the solution even at concentrations below 0.1 *N*, and many salts had a pronounced depressing effect at higher concentrations of 1.0 to 2.0 *N*. These inhibitory effects were in good agreement with those observed in extraction, as can be seen from the "maximum" alpha-amylase values. For some salts, sodium chloride for instance, the "maximum" extraction activities were not obtained, although they showed no depressing effect on the solution. However, sodium chloride gave "maximum" extractions when employed on some other types of malts, wheat malt for instance, and also when the time of extraction was increased, as will be shown later.

For beta-amylase the position is a different one, especially at concentrations below about 0.5 *N*. Some salts, such as bicarbonates and acetates, somewhat stimulated the activity of the solution, and this influence appeared to be reflected in corresponding increases of the extraction figures. Higher concentrations were predominantly depressing, but in most instances differently than for alpha-amylase.

Effect of Natural Waters and of Papain on the Extraction of Barley Malt Amylases. It was of interest to compare the effects of salts

TABLE III

COMPARATIVE EFFECTS OF SALTS ADDED BEFORE AND AFTER
EXTRACTION ON AMYLASE ACTIVITIES OF EXTRACTS¹

Salt	Con- centra- tion	Alpha-amylase activity				Beta-amylase activity			
		20°C.		30°C.		20°C.		30°C.	
		Before extr.	After extr.	Before extr.	After extr.	Before extr.	After extr.	Before extr.	After extr.
	N	%	%	%	%	%	%	%	%
Sodium chloride	0.05	85	100	85	—	100	100	106	—
	0.5	85	100	85	—	100	100	100	—
	1.0	90	100	85	—	100	100	100	—
	2.0	70	—	70	(85) ²	—	—	99	(90)
Sodium nitrate	0.05	90	100	90	100	100	100	100	100
	0.5	95	100	90	—	100	95	100	—
	1.0	90	100	95	90	95	90	90	85
	2.0	90	—	90	(85)	95	—	90	(90)
Sodium acetate	0.05	90	—	90	105	105	—	105	105
	1.0	85	—	85	90	90	—	90	90
	2.0	55	—	60	(70)	70	—	75	(75)
Sodium bicarbonate	0.05	90	—	95	100	100	—	105	115
	1.0	85	—	90	75	90	—	90	95
	2.0	65	—	55	(70)	90	—	90	(80)
Sodium citrate	0.2	90	90	—	—	100	110	—	—
	1.0	65	—	—	—	90	—	—	—
Potassium dihydrogen phosphate	2.0	35	—	0	(0)	105	—	100	(105)
Diammonium hydro- gen phosphate	2.0	75	—	75	(85)	70	—	75	(75)
Calcium acetate	0.01	70	95	75	—	100	100	100	—
	0.05	80	—	85	80	100	—	105	105
	0.2	85	80	85	—	100	110	100	—
	1.0	75	—	80	70	95	—	90	90
	2.0	70	—	75	(80)	90	—	90	(90)
Calcium chloride	0.05	75	—	85	95	100	—	100	100
	1.0	75	—	80	85	95	—	95	30
	2.0	55	—	55	(65)	14	—	0	(8)
Magnesium sulfate	0.05	85	95	85	90	95	105	100	100
	0.5	85	90	85	—	95	100	100	—
	1.0	65	85	60	65	95	100	95	100
Sodium sulfocyanate	0.05	95	—	95	105	95	100	95	100
	1.0	80	—	50	25	45	—	30	5
Potassium iodate	0.05	0	—	0	0	0	—	0	—

¹ Extractions for 2 hours at 20° and 30°C. Amylase determinations in extracts at 20°C. Alpha-amylase for salts added *before* extraction in per cent of respective "maximum" extraction values; all other activities in per cent of "distilled water" values.

² Values in parentheses were measured on amylase solutions obtained after extracting one wheat malt (see below).

added to the distilled water before extraction with the influence of natural or industrial waters upon the extraction of malt.

A brewers' malt was extracted with: (a) distilled water, (b) a city water containing 280 p.p.m. total minerals mostly in the form of calcium and magnesium bicarbonate (average normality of 0.007), (c) a well water containing 1,350 p.p.m. total minerals, about one-half of which were bicarbonates and the remainder mainly calcium sulfate (average normality 0.027), (d) sodium bicarbonate and calcium acetate solutions of the same normalities as the two samples of water, and (e) 1% papain solution in water and in 1.0 *N* calcium chloride

TABLE IV

EFFECT OF EXTRACTING BREWERS' MALT WITH NATURAL WATERS AND WITH PAPAIN IN DISTILLED WATER ON AMYLASE ACTIVITIES¹

Extraction medium	Alpha-amylase		Diastatic power		Beta-amylase		pH of extract
	20°C.	30°C.	20°C.	30°C.	20°C.	30°C.	
Distilled water	58	74	100	102	100	98	5.5-5.8
Calcium acetate, 0.007 <i>N</i>	67	76	102	104	100	101	6.6
City water, 0.007 <i>N</i>	78	89	107	121	102	114	6.4-6.7
Sodium bicarbonate, 0.007 <i>N</i>	82	91	110	111	106	103	6.8
Sodium bicarbonate, 0.01 <i>N</i>	85	100	110	113	103	103	7.0
Sodium bicarbonate, 0.027 <i>N</i>	89	97	108	115	102	106	7.4
Well water, 0.027 <i>N</i>	79	89	113	117	108	109	6.7
Calcium acetate, 0.027 <i>N</i>	78	84	106	109	101	102	6.7
Papain, 1% emuls.	79	80	126	132	122	130	5.3-5.5
Papain in 1.0 <i>N</i> calcium chloride	79	79	124	119	120	115	5.3
Calcium chloride, 1.0 <i>N</i>	77	80	102	101	96	93	6.3

¹ Extraction of brewers' malt "A" for 2 hours at 20° and 30°C. Amylase determination in extracts at 20°C. Alpha-amylase is expressed in per cent of "maximum" activity, diastatic power and beta-amylase in per cent of "distilled water" values.

solution respectively. The results of pH and amylase activity determinations are recorded in Table IV.

The alpha-amylase activities of both natural water extracts fell between those of the two salts. For the city water they were closer to bicarbonate, whereas for the well water they were closer to those for the calcium salt; this agrees with the respective contents of these mineral salts. For beta-amylase, a slightly higher stimulation was found than would be expected on the basis of the bicarbonate content.

With a 1% papain extraction, the beta-amylase activity was, as expected, much more strongly increased than by any salt. For alpha-amylase the picture is an entirely different one: only about 80% of the "maximum" value was reached. When 1% papain was added to a

1.0 *N* calcium chloride solution, the alpha-amylase activity of the latter was not changed, but the beta-amylase was strongly increased close to but somewhat below that of the papain-distilled water emulsion, the calcium chloride exerting its slight depressing effect. This may indicate that commercial papain improves the release of alpha-amylase only by adding soluble salts to the extraction solution, but without any release from a bound or inactive form as is well known to be the case for beta-amylase.⁴

Effect of Temperature and Time of Extraction. Further experiments were conducted at an extraction temperature of 40°C. using the same

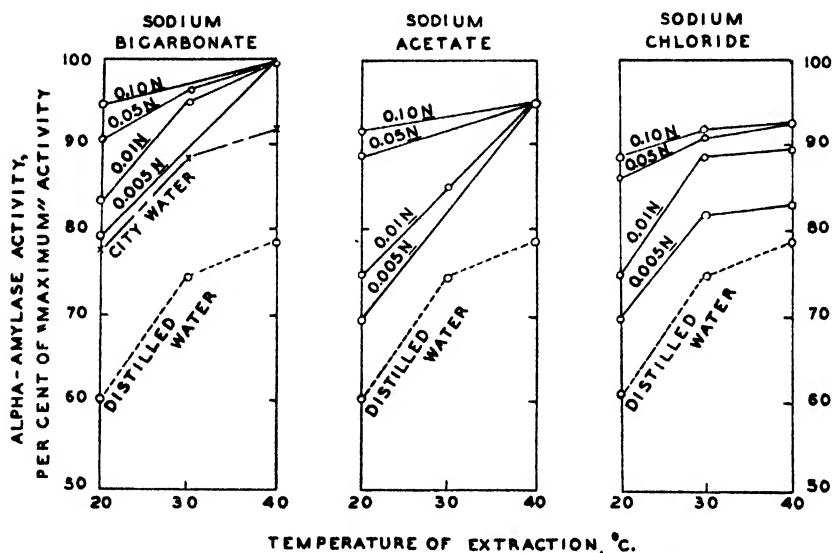


Fig. 2. Effect of temperature on the extraction of alpha-amylase of brewers' malts, at various salt concentrations. Extraction for two hours at 20° and 30°C., for one hour at 40°C.

series of salt concentrations employed in previous extractions at 20° and 30°C. The amylase activities were measured at 20°C.

The alpha-amylase activities were higher than at 30°C. in the lowest concentration bracket. Optimum activities were again obtained below or at about 0.1 *N*, but no activity was found to exceed the "maximum" alpha-amylase determined for the respective malts at 30°C. with suitable salts. Only with salts such as sodium chloride, which did not yield these "maximum" values at the lower temperature, were improvements noted. Different barley malts responded similarly to the increased temperature of extraction.

⁴ Subsamples of the papain (Merck), extracted for 2 hours at 20° and 30°C. with distilled water, gave an average of 70.6% total soluble matter and 8.7% soluble mineral ash. This means that the filtered extract contained 0.087% inorganic minerals (ashed) coming from the papain, corresponding to an approximately 0.02 *N* salt solution (see Table II).

This is illustrated in Fig. 2. Sodium bicarbonate is an example of salts which gave the "maximum" alpha-amylase activities, at 0.1 *N*, at 20° and 30°C. within a margin of about 5%. Sodium acetate reached the "maximum" very closely at the lower temperatures, but completely at 40°C. even at the lowest salt concentrations. Sodium chloride remained below the "maximum" at 40°C., particularly at suboptimal salt concentrations. The respective values obtained with "city water" of 0.007 *N* were between those of sodium chloride and sodium bicarbonate. "Distilled water" acted as the very dilute salt solution it actually forms during the extraction of malt.

A similar picture resulted when the duration of extraction was increased. Sodium bicarbonate gave the "maximum" alpha-amylase value at 30°C. after about 1 hour, and no further increase up to 4 hours. It yielded the same "maximum" when the extraction was accelerated by agitation, in a Waring Blendor, after 5 to 10 minutes at 40°C. with no further increase in 15 and 20 minutes. Extraction with a 1% papain emulsion gave about 80% of the maximum alpha-amylase activity after 2 hours at 20°C., and 85 to 95% after 22 hours, depending on the malts tested, whereas extraction with 0.1 *N* sodium chloride for 22 hours at 40°C. gave 95 to 100% of the "maximum."

For beta-amylase, the temperature of 40°C. gave signs of slight destruction, especially when agitation was applied.

Variations of the pH of the extract from about 5 to 8 did not appear materially to alter either the alpha- or beta-amylase activities. Higher acidity or alkalinity caused the well-known differential effects with alpha- and beta-amylase. For purposes of illustration, results are recorded in Table II for citric acid, primary phosphate, and calcium hydroxide. Although calcium hydroxide is an extracting agent similar to other calcium compounds, it caused a small loss in alpha-amylase activity at pH 11, whereas the beta-amylase activity was already badly affected at pH 8.5. Conversely, citric acid destroyed alpha-amylase activity completely below pH 4.5, but only part of the beta-amylase activity.

Oxidizing agents have a destructive effect upon both amylases, but it is more pronounced for beta-amylase activity. Calcium peroxide, for instance, increased the alpha-amylase activity about the same degree as did calcium hydroxide, but destroyed beta-amylase at lower pH values. These and other data for ammonium persulfate and potassium periodate are recorded in Table II. Any possible influence of such chemicals upon the starch reagent (4, 5, 44) and upon the ferricyanide titration was taken into account by adequate blank tests.

The effect of reducing salts, such as sulfocyanate, had been studied in connection with starch hydrolysis (6, 12). Sodium sulfocyanate was

found to be a good extracting agent for alpha-amylase, but had no apparent activating influence upon beta-amylase (Tables II and III). In concentrations higher than 0.2 *N*, it caused destruction of both amylases.

Influence of Various Salts on the Extraction of Amylases from Wheat Malt. The effects on amylase activity of extracting wheat malt with various solutions at different temperatures are recorded in Table V. Although this study was not sufficiently extensive to permit definite conclusions for wheat malts in general, it was of interest to compare the results with those described above for barley malt.

For alpha-amylase the results were closely similar to those for barley malt; "maximum" activities were reached within the same range of salt concentrations.

TABLE V
EFFECT OF EXTRACTING WHEAT MALT WITH VARIOUS
SOLUTIONS ON AMYLASE ACTIVITIES¹

Extraction medium	Salt concentration	Alpha-amylase activity extracted at			Beta-amylase activity extracted at		
		20°C.	30°C.	40°C.	20°C.	30°C.	40°C.
Distilled water	A	84	89	94	100	104	105 ²
		79 ³	94 ³	—	100 ³	122 ³	131 ³
		—	—	—	—	—	—
City water	—	89	95	—	109	112	—
Papain, 1%	—	89	—	—	247	—	—
		88 ³	—	86 ³	237 ³	—	288 ³
Sodium bicarbonate	0.1	95	100	94	158	164	160
		100 ³	100 ³	100 ³	—	163 ³	—
	0.5	95	95	94	—	—	—
		94 ³	94 ³	—	—	—	—
	2.0	95	80	—	100	87	—
Sodium chloride	0.1	100	100	94	155	167	167
		88 ³	100 ³	—	169 ³	174 ³	—
Sodium acetate	0.1	89	89	—	144	149	—
Calcium chloride	0.1	84	95	—	148	148	—
	0.5	73	80	—	93	92	—
	2.0	53	53	—	0	0	—

¹ Extraction for 2 hours at 20° and 30°C., for 1 hour at 40°C. Amylase determinations in extracts at 20°C. Alpha-amylase calculated in per cent of "maximum" activity, beta-amylase in per cent of "distilled water" values.

² Wheat malt "D" having a "maximum" alpha activity of 33 "20° dextrinizing units," a diastatic power of 106°L.

³ Wheat malt "E" having a "maximum" alpha activity of 18 "20° dextrinizing units," a diastatic power of 57°L.

TABLE VI
RELATIONSHIP BETWEEN "MAXIMUM" AND "DISTILLED WATER"
ALPHA-AMYLASE VALUES FOR DIFFERENT MALTS

Nature of malt ¹		Moisture of sample	Diastatic power	Alpha-amylase ²		
				"Maximum" dextrinizing units	"Distilled water"	
					Dextri- nizing units	Per cent of "maxi- mum"
		%	°/.			
Barley brewers'	1	3.9	96	28	16	57
	2a	4.5	127	33	21	64
	2b	4.4	136	39	25	64
	3a	5.0	141	32	20	63
	3b	5.0	149	36	21	58
	4	4.6	125	31	17	55
	5	4.0	110	36	21	58
	6	4.0	121	29	18	62
	7	4.8	146	36	23	64
Barley distillers'	8a	6.3	184	47	23	49
	8b	6.4	179	46	23	50
	8c	5.5	161	40	27	68
	8d	5.8	163	43	28	65
	9a	6.5	196	44	24	55
	9b	6.2	193	43	21	49
	9c	6.1	182	43	20	47
	9d	6.3	194	43	22	51
	9e	7.1	190	47	24	51
	9f	6.7	192	49	29	59
	9g	6.5	184	47	26	55
	10	6.2	182	32	18	56
	11	6.8	188	38	22	58
	12	6.1	232	32	16	50
	13	7.9	186	30	21	70
	14	6.4	191	47	29	62
Barley green ³	15	8.7	—	35	27	77
	16	7.2	—	47	35	74
	17	7.4	148	33	19	58
	18	7.6	219	52	27	52
Wheat malt	19	11.4	57	18	14	78
	20	7.9	106	33	27	82

¹ Samples designated with the same number but with different letters represent malts of the same shipment but processed under different conditions.

² "Maximum" alpha-amylase activity determined by extracting with 0.1 N sodium bicarbonate at 30°C. for 2 hours; "distilled water" activity by extracting at 20°C. for two hours, activities in extracts measured at 20°C.

³ Dried in vacuo.

For beta-amylase, however, small additions of salts increased the diastatic power much more than for barley malts, thus indicating the presence of considerable amounts of salt-soluble beta-amylase.

Higher salt concentrations gave decreases for both amylases, as had been found with barley malt. The effect of salt additions to wheat

amylases in solution was strikingly similar to that shown for the barley malt amylases (see Table III).

Relationship between the "Maximum" Alpha-Amylase Activities and the "Distilled Water" Activities of Different Malts. From the foregoing studies, it appeared that one "maximum" alpha-amylase activity could be determined by extracting any malt with (0.05 to) 0.1 *N* sodium bicarbonate solution at 30°C. for (1 to) 2 hours. These "maximum" activities were determined on a wide variety of malts comprising barley brewers', distillers', and green malts, and two wheat malts. For each sample the "distilled water" activity was also determined after extraction at 20°C. for 2 hours. The results are shown in Table VI.

For the nine brewers' malts, the distilled water values for alpha-amylase activity only varied from 55 to 64% of the corresponding "maximum" values. For the other types of malts, however, a much wider spread (47 to 82%) was found.

Discussion

The release of *alpha-amylase* from barley and wheat malt is extremely sensitive to the presence of small amounts of salts. When malts are extracted with distilled water, it must be considered that the grain minerals are partly water-soluble. In the usual 5% malt infusion, one-tenth of the ash content of 2 to 3% is sufficient to add about 100 p.p.m. of mineral salts to the water, and the extraction would actually be made with a 0.001 to 0.002 *N* salt solution. Quantitative and qualitative variations of the grain ash, depending on the grain variety and on the method of malting and drying, can cause differences in the alpha-amylase activities measured in distilled water extracts. It has indeed been reported that more concentrated malt mashes give higher amylase activities (19, 43). It remains to be seen whether the presence of traces of electrolytes is essential for the activity of cereal alpha-amylase, as seems to be the case for alpha-amylases of other origin (9, 29, 34).

Alpha-amylase activities of barley malts show some correlation with their calcium and magnesium content (22), and this activity is also distinctly higher in mill-stream wheat flours which are richer in ash (7). In such cases, the grain minerals may have influenced, at least partly, the activity of the distilled water extracts used for the analyses.

When salts are added to the extraction water, the influence of small variations in the mineral content of the sample disappears. This is particularly true at the concentration of about 0.1 *N*, where optimum alpha-amylase activities were found for all salts examined, and "maxi-

imum" activities with certain salts. It has been reported that barley malts extracted with a 0.4 *N* sodium chloride solution gave in the filtrates alpha-amylase activity the same as or slightly lower than the infusion of these malts in distilled water without filtration (43). In this study such salt extractions yielded extracts which showed 85 to 100% of the "maximum" activities. The increases in alpha-amylase caused by salt additions have been reported to vary in a wide range, up to 82% of the "distilled water" extracts (19, 24, 27); in the present study increases up to 110% were found (Table VI).

The extraction conditions which gave the "maximum" alpha-amylase activities approximate those which prevail in breweries and distilleries. Bicarbonates and sulfates are usually the predominating anions, and calcium and magnesium the principal cations contained in industrial mash waters. Such waters (see "city water" in Fig. 2) gave, at 40°C. after 1-hour extraction, 90 to 95% of the "maximum" activity of the respective malt.

These results do not constitute definite proof that the "maximum" values are the true potential alpha-amylase activities, but they may be more representative for each malt than those obtained with other extractions, especially with distilled water.

The release of the "latent" *beta-amylase* from malts is significantly different from the effect of inorganic salts on alpha-amylase. This is clearly apparent when the action of papain on alpha- and beta-amylase is compared. Whereas its effect on beta-amylase exceeds by far that of any salt, the reverse is true with alpha-amylase. With the latter, no proteolytic actions or reactions involving the sulfhydryl group of the enzyme molecule were apparent. In barley and wheat grains part of the latent beta-amylase can be activated by salts (14, 15, 17, 33). Such salt-soluble beta-amylase was not found in any substantial amounts in the barley malts here examined, but very large amounts were determined in two wheat malts. This is reflected in the effect of small salt additions upon the total saccharifying activity. It is known that salts cause relatively small increases of diastatic power in barley malts (3, 10, 13, 27); the influence is, however, considerable for wheat malts.

Concurrent with the release of the amylases from malt during extraction, salts also affect the activities of both *enzymes in their water solutions*. In low concentrations, only beta-amylase was found to be stimulated by some salts. At higher salt concentrations, a pronounced depressing action upon the activity of the enzyme in solution overshadows more and more the effect of salts on the extraction of the amylase enzymes. These depressing effects are specifically different for the individual ions, and are not identical for alpha- and beta-

amylase. No marked variation was found, however, between barley and wheat malt amylases. This should be noted in connection with reports describing the conditions for the differential stability of the two amylases in wheat, on the one hand, and in barley malt, on the other (18, 21).

The general effects of the different ions in 2.0 *N* solution (pH 5 to 8) upon the activities of the two amylases in solution are summarized in Table VII.

TABLE VII
EFFECT OF INDIVIDUAL IONS ON ALPHA- AND BETA-AMYLASE
IN WATER SOLUTION, WITHIN A pH RANGE OF 5 TO 8

Ions in solution	Alpha-amylase		Beta-amylase	
	Ion concentration		Ion concentration	
	Approx. 0.1 <i>N</i>	1.0-2.0 <i>N</i>	Approx. 0.1 <i>N</i>	1.0-2.0 <i>N</i>
Sodium	0 ¹	—	0	0
Potassium	0	0	0	0
Ammonium	0	—	0	—
Calcium, magnesium	—	—	0	—
Chloride	0	—	0	—
Nitrate	0	0	0	—
Acetate	0	—	+	—
Sulfocyanate	0	—	0	—
Sulfate	0	—	0	0
Bicarbonate	0	—	+	0
Phosphate	0	—	+	+
Citrate	—	—	+	—

¹ "0" means: No significant influence.

"—" means: Activity slightly decreased.

"——" means: Activity more decreased.

"———" means: Activity strongly decreased.

"+" means: Activity slightly stimulated.

These approximate indications agree in many, but not in all, instances with work already published. Previously (12, 37, 38, 39), only the saccharogenic activities had been measured and compared for both amylases. Other differences may be explained by the fact that in the present study the amylase solutions were strongly diluted before the measurement of their activities, in accordance with usual practice. When the starch hydrolysis is conducted in the presence of salts in much higher concentrations (12), the results may be different.

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INFLUENCE OF SMALL QUANTITIES OF THIAMINE ON. BAKING QUALITY OF WHEAT FLOUR¹

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ABSTRACT

The influence of small quantities of thiamine on the baking quality of flour was investigated with the aid of extensograph, fermentograph, and baking experiments, using flours differing both in extraction and in the composition of the grist from which they were milled.

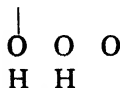
Thiamine added in concentrations up to 12 mg. per kg. of flour had no influence on the elastic properties of dough. In concentrations up to 10 mg. per kg. of flour it, apparently, exercised a slight stimulating action on the fermentation.

Even when variations were made in the type of flour, yeast concentration, dough consistency, and fermentation time, it was not possible to reproduce the improvement in the bread volume of 6.8% and 15.7% claimed in Dutch patent No. 55774 for the addition of 3 and 6 mg. respectively of thiamine per kg. of flour.

In the concentrations used, it is concluded that thiamine does not possess any improving action in breadmaking.

It is well known that very small concentrations of various oxidizing substances, such as potassium bromate, potassium iodate, and ammonium persulfate, exercise an improving influence on the baking quality of wheat flour. The extensive investigations and the many theories evolved to explain the behavior of these substances have been summarized by Sullivan *et al.* (8) and by Shen and Geddes (7). The thesis of Holger Jørgensen (3) is also a worth-while contribution to the elucidation of this problem.

Certain organic reducing agents such as ascorbic acid, reductic acid, and related substances, which, together with the oxidized form, constitute a redox-system can exercise a similar action to the above-mentioned oxidizers. From experiments in which ascorbic acid and a number of substances of analogous structure were compared as to their effect on the baking quality of wheat flour, Elion (1) was of the opinion that the group —C=C—C— performs an essential function in the improve-



ment of baking quality. Sandstedt and Hites (6) showed that the presence of the specific reducing group only fulfills one requirement.

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A second condition is that the oxidases in the flour must be able to form a redox-system from the reducing substance and its oxidized form. This system catalyzes the transfer of oxygen contained in the dough to the oxidizable groups in the protein components of the flour, where the oxidation by bromate and similar substances also takes place. Thus the action of both groups of substances can be reduced to the same principle. Maltha (4) has arrived at a similar point of view.

Regarded in this light, it is rather surprising to find in a patent by Hoffmann-La Roche (2) an improvement of baking quality attributed to thiamine, a chemically different type of substance, which does not possess the above-mentioned characteristic properties. According to the specification the addition of 3 and 6 mg. thiamine per kg. of flour exerts a volume improvement of 6.8 and 15.7% respectively.

Moran and Drummond (5), investigating the properties of white flour enriched with thiamine, did not notice any influence on the dough, the volume of the loaf, or the crumb character of the bread.

Because of the importance of the patent from a social and economic point of view the author has tried to reproduce the claimed improvement. Although comparison is made in the patent to an action such as that of bromate and ascorbic acid—in other words, the influencing of the protein-proteinase system—attention was paid in the present studies to the possible activation of fermentation by the added thiamine, which could likewise cause an increase in bread volume.

Materials and Methods

A rather wide choice of flours was employed in case some types would be more susceptible than others to any effect produced by the thiamine.

a. A flour milled in a Dutch flour-mill from a grist consisting of 40% home-grown wheat, 40% foreign wheat, and 20% rye,³ from which were separated the fractions 0–30% (patent flour A₁) and 30–67% (clear flour B₁). By mixing the fraction 0–30% with that of 30–67% in the proportion of 3:3.7, a flour (AB₁) was obtained having an extraction of 67%, which is not far short of the prewar standard in Holland.

b. A flour milled by the same mill, from a mixture consisting of 50% foreign wheat, 30% home-grown wheat, and 20% rye. The same fractions 0–30% (A₂) and 30–67% (B₂) were separated, and by mixing these fractions in the proportion 3:3.7, a flour (AB₂) of 67% extraction was compounded.

c. A flour milled exclusively from soft home-grown wheat to an extraction of about 75%.

³ In the present situation of world wheat-shortage the millers are bound by the government to admit a certain amount of rye, mostly home-grown, to the grist.

d. A national bread-flour: "Type 10 November 1946." Composition: 50% foreign wheat, 30% home-grown wheat, and 20% rye, and extraction of 85%.

The yeast used was furnished by the Ned. Gist en Spiritusfabriek (Koningsgist).

Sodium chloride (a highly purified product) was supplied by Brocades Stheemann and Pharmacia, Meppel, and thiamine (synthetic) by the "Chemo Puro Manufacturing Company," New York.

The effect of thiamine on the protein- (gluten-) system was studied with the aid of the Brabender Extensograph, since the nature of the extensogram is known to be markedly influenced by small concentrations of such improving agents as potassium bromate and ascorbic acid. The procedure was as follows: 300 g. flour plus 6 g. sodium chloride and the thiamine, when used, were kneaded with water in the Brabender Farinograph to the normal consistency of dough (500 Brabender units). Each piece of dough was divided into two parts of 150 g. These were worked and rolled into the desired shape and set aside at a temperature of 27°C. At intervals of 45 minutes four extensograph curves were made for each piece of dough.

The influence of fermentation activity was examined with the aid of the Brabender Fermentograph as follows: The dough, prepared from flour, 2% yeast, 2% common salt, and the thiamine when used, was kneaded in the farinograph to a consistency of 450 to 500 Brabender units. Immediately after preparation an accurately weighed quantity (400 g.) was placed in the fermentograph at a temperature of 27°C. At intervals of one hour the piece of dough was well punched in accordance with the process in the bakery. The total duration of the experiment was from 4 to 5 hours; that is, about the time when the fermentation begins to weaken. With only a few exceptions, each experiment was carried out in duplicate.

The influence of thiamine on the properties of the bread was investigated by several series of baking tests. Six tinned loaves of 400 g. (each containing about 240 g. of dry substance) were baked from each dough. A dough temperature of 26° to 27°C., a proofing temperature of about 30°C., and an oven temperature of 260° to 270°C. were always maintained. Ingredients to promote diastatic power were not added, since the composition of the grain mixtures insured sufficient enzyme activity. Thiamine was added to the dough-liquor in the form of a concentrated solution by means of a pipette. Variations were introduced in the percentage of yeast, consistency of the dough, and the length of fermentation to discover the conditions most favorable to the action of thiamine on the baking behavior. The volumes of the loaves

were determined immediately after baking, and the remaining properties were estimated after about 18 hours.

Results and Discussion

The extensograph data for the 45- and 180-minute curves are summarized in Table I. Besides the highest concentration of thiamine mentioned in the patent, i.e., 6 mg. per kg. flour, an addition of 12 mg. was also examined, the greater amount thus giving a possibly vague influence of 6 mg. a chance to become evident.

For the same reason, concentrations of 10 mg. thiamine per kg. flour, as well as those of 6 mg. per kg. were examined in the fermentation experiments. Variations were also made in dough consistency and percentage of yeast. The results are summarized in Table II.

Finally the results of the different series of baking tests are collected in Table III.

The extensograms (Table I) show in a striking manner that thiamine has no effect upon the stretching properties of the dough. The curves with and without the addition of thiamine are the same at each of the

TABLE I
EFFECT OF THIAMINE ON EXTENSOGRAM MEASUREMENTS

Property measured	After 45 minutes			After 180 minutes		
	Thiamine, mg./kg. flour			Thiamine mg./kg. flour		
	0	6	12	0	6	12
FLOUR A ₂ (0-30% EXTRACTION)						
Water absorption—%	52.5	52.5	52.5	52.5	52.5	52.5
Extensogram area—cm. ²	90.5	90.6	83.2	103.0	109.8	107.4
Max. stretching power—B. U.	370.0	365.0	340.0	540.0	545.0	540.0
Extensibility—cm.	17.5	16.6	17.0	14.4	14.7	14.5
FLOUR B ₂ (30-67% EXTRACTION)						
Water absorption—%	53.5	53.5	53.5	53.5	53.5	53.5
Extensogram area—cm. ²	73.7	77.1	67.0	83.7	88.7	75.5
Max. stretching power—B. U.	310.0	310.0	300.0	420.0	420.0	385.0
Extensibility—cm.	16.5	16.5	16.2	14.2	14.5	14.2
FLOUR AB ₂ (0-67% EXTRACTION)						
Water absorption—%	52.5	52.5	52.5	52.5	52.5	52.5
Extensogram area—cm. ²	82.1	84.1	83.5	100.3	103.6	99.8
Max. stretching power—B. U.	340.0	340.0	335.0	490.0	505.0	495.0
Extensibility—cm.	16.5	17.0	17.1	14.1	13.3	14.2

TABLE II

EFFECT OF THIAMINE ON THE GAS PRODUCTION OF DOUGHS OF VARYING CONSISTENCY AND YEAST CONTENT, MADE WITH FLOURS OF DIFFERENT COMPOSITION AND EXTRACTION¹

Flour	Extractions	Yeast	Thiamine	Absorption	Farinograph consistency	Total gas production ²	
						4 hours	5 hours
	%	%	mg./kg.	%	B. U.	ml.	ml.
A ₁	0-30	2	Control	50	500	1400	—
		2	10	50	500	1440	—
AB ₁	0-67	2	Control	52	450	1480	—
		2	6	52	450	1540	—
AB ₁	0-67	2	Control	51	500	1475	—
		2	10	51	500	1515	—
AB ₁	0-67	1	Control	53	450	765	1075
		1	6	53	450	900	1180
AB ₂	0-67	2	Control	53	450	1505	1955
		2	6	53	450	1525	1980
AB ₂	0-67	1	Control	53	450	790	1110
		1	6	53	450	835	1165

¹ All dough contained 2.0% of salt (flour basis). Mean values of duplicate determinations.

² The mean error of the mean values is about 80 ml.

testing times so that an action similar to that of bromate, ascorbic acid, or similar improvers cannot be ascribed to thiamine.

The figures derived from the fermentographic investigation (Table II) are inconclusive. The indicated influence of the thiamine on the fermentation is slight and of the same magnitude as the mean error of the mean of the duplicate determinations, but the fact that in all six series of experiments the addition of thiamine gave an increased gas-production suggests that thiamine has a positive influence on the fermentation. However, statistical calculations indicate that this influence is so slight that no definite conclusions can be drawn from it.

The question arises whether the bakers' yeast used in Switzerland, with which the experiments described in the patent in question were probably carried out, contained less thiamine than the Dutch yeast and in consequence was more sensitive to additions of this substance. However, the manufacture of bakers' yeast in West European countries is essentially the same, thus making important differences in composition very unlikely.

The baking experiments confirm the results of the dough investigation. The differences in volume of the loaves made with and without thiamine were at the most 1.5%, thus falling within the limits of experimental error. An apparent decrease in volume due to the addition of thiamine appeared in the series with the flour milled from Dutch soft wheat.

To conclude the investigation, a commercial baking test was carried out, to determine whether any differences could be obtained in the commercial bakery which uses sacks of 50 kg. of flour. Bread baked from a sack of government flour (50% foreign wheat, 30% home-grown wheat, 20% rye: 85% extraction) with thiamine added at the rate of 6 mg. per kg. flour gave similar loaves to that baked without thiamine.

TABLE III

EFFECT OF THIAMINE ON THE BAKING BEHAVIOR OF DIFFERENT FLOURS WITH VARIATIONS IN PERCENTAGE OF YEAST AND FERMENTATION TIME¹

Procedure					Baking results				
Thiamine added per kg. flour	Yeast	Absorption	Total fermentation time	Total proof time	Shape	Texture	Loaf volume (mean)	Volume per kg. flour	Volume increase
mg.	%	%	min.	min.			cc.	cc.	%
FLOUR A ₂ (0-30% EXTRACTION)									
Control	2	55	43	83	Good	Good	1720	6005	—
3	2	55	43	83	Good	Fair	1740	6070	+1.1
6	2	55	43	83	Good	Satisfactory	1740	6070	+1.1
Control	1	55	53	98	Satisfactory	Satisfactory	1530	5330	—
3	1	55	53	98	Satisfactory	Satisfactory	1530	5330	0
6	1	55	53	98	Satisfactory	Satisfactory	1530	5330	0
12	1	55	53	98	Satisfactory	Satisfactory	1550	5370	+0.8
FLOUR AB ₂ (0-67% EXTRACTION)									
Control	2	55	40	93	Good	Fair	1590	5540	—
3	2	56	40	93	Good	Fair	1590	5540	0
6	2	56	40	93	Good	Fair	1590	5540	0
Control	1	56	50	103	Satisfactory	Satisfactory	1390	4810	—
3	1	56	50	103	Satisfactory	Satisfactory	1390	4810	0
6	1	56	50	103	Satisfactory	Satisfactory	1410	4880	+1.4
SOFT DUTCH FLOUR (75% EXTRACTION)									
Control	2	57.5	40	82	Moderate	Fair	1100	3760	—
3	2	57.5	40	82	Moderate	Fair	1080	3690	-2
6	2	57.5	40	82	Moderate	Fair	1095	3745	-0.5
12	2	57.5	40	82	Moderate	Fair	1085	3710	-1.5

¹ Added sodium chloride 2% (flour basis); baking time 30 minutes.

The experimental results given here show that it has not been possible to reproduce the improvement in volume of 6.8 and 15.7% respectively, on addition of 3 and 6 mg. thiamine per kg. flour, claimed in the patent in question. This applies to flour made from different grists, milled to different extractions, and baked by different formulas.

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BOOK REVIEWS

Techniques of Histo- and Cytochemistry. By David Glick. 555 pp. Interscience Publishers, New York, N. Y. 1949. Price \$8.00.

As stated in the flyleaf, this book is "A Manual of Morphological and Quantitative Methods for Inorganic, Organic, and Enzyme Constituents in Biological Materials." The material presented is grouped in four parts, Microscopic Techniques, Chemical Techniques, Microbiological Techniques, and the Mechanical Separation of Tissue Components. The first two parts comprise over 90% of the text. The author has been commendably successful in bringing together in one volume the diverse methods that will serve as a very valuable aid to the research worker whose problems make it necessary to evaluate small samples of biological materials, in particular, to localize or estimate substances in the components of cells.

Appraisals and limitations of many of the procedures are given, but most of the methods are presented without evaluation. It is the author's apparent intention to describe the methods in detail and allow the investigator to use his own judgment in their application. Brief surveys of the literature relevant to the various techniques are included, together with an ample and useful bibliography. For most of the procedures the user will want to consult the original publications on the method and related subjects, and this distracts somewhat from the usefulness of the presentation of isolated, detailed instructions.

The difficult task of organizing and presenting the material is satisfactorily handled, although room for improvement is evident. For example, a historical discussion on diffusion and titrimetric methods for nitrogen and ammonia falls in the section on cuvette colorimetry. In the section on the isolation of cell nuclei, in variance with the bulk of the book and somewhat confusing to the reader, the introductory discussions are given in small print at the end of the previously presented method.

The microscopic techniques described are "those designed to establish the distribution of elements, groups, substances, or activities in microtome sections of tissues by means of examination under some form of a microscope." The section on chemical methods is prefaced by a useful discussion of the requirements and limitations of the methods. Techniques applicable to the localization of some 20 inorganic elements or radicals, 28 organic substances and groups, and 11 enzymes are presented. The section on physical methods includes techniques of fluorescence microscopy, emission and absorption histospectroscopy, microincineration, electron microscopy, and radioautography.

The material presented under the general heading of chemical techniques could be profitably consulted by anyone interested in microchemical methods. General apparatus and microchemical manipulations are described. Colorimetric methods which make use of the capillary tube technique and the microcuvette technique are

presented, followed by a good section on titrimetric techniques. The gasometric techniques described include those for micro gas analysis and for microrespirometry. Sections on dilatometric techniques and the determination of the amount of a biological sample comprise the other important portions.

The part devoted to microbiological techniques is limited to a presentation of an adaptation of the procedure for riboflavin to small volumes. The possibilities for further development in this field are deservedly mentioned.

The part on mechanical separation of cellular components is devoted to centrifugal and associated techniques for the isolation of cell nuclei, chromatin threads, cytoplasmic particles, and chloroplasts. It is these techniques together with the chemical and physical methods of analysis which R. R. Bensley in a foreword to the book regards as the "new microchemistry," which "promises to become the most important tool we possess for elucidation of the fundamental chemical pattern of protoplasm."

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Carl Alsberg—Scientist at Large. Edited by Joseph S. Davis. 182 pp. Stanford University Press, Stanford, California. 1948. Price \$2.00.

Many cereal chemists will remember Carl Alsberg as one-time associate editor of this journal, as one who shared in the work of deciding the conditions of the Osborne Medal Award, and as an active worker in the field of cereal chemistry. However, Dr. Alsberg's interests extended far beyond cereal chemistry, ranging from the natural to the social sciences and the study of international relations. He was successively head of the Bureau of Chemistry, Department of Agriculture, a director of the Food Research Institute at Stanford University, and director of the Giannini Foundation for Research in Agricultural Economics at the University of California.

This book is edited by J. S. Davis who collaborated with Dr. Alsberg at the Food Research Institute, Stanford University. Five of Dr. Alsberg's former colleagues, A. L. Kroeber, D. D. Van Slyke, F. B. Linton, R. D. Calkins, and J. B. Condliffe, have each written a biographical chapter covering the various aspects of his career.

These chapter titles are, respectively, *The Making of the Man*, *Work in the Natural Sciences*, *Chief of the Bureau of Chemistry*, *University Professor and Administrator*, and *Social Scientist Beyond the University*. Besides these fine tributes to an outstanding man, the book contains three of Dr. Alsberg's own stimulating papers, namely, "Progress in Chemistry and the Theory of Population," "What the Social Scientist Can Learn from the Natural Scientist," and "Commencement Address at Reed College."

The book also includes a classified bibliography of Alsberg's publications.

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Biochemical Preparations. Volume I. Edited by Herbert E. Carter. XI + 76 pp. John Wiley and Sons, Inc., New York, N. Y. 1949. Price \$2.50.

This is the first of a projected series of volumes describing the preparation of compounds of biochemical origin or interest. It is the aim of the editorial and the advisory boards to cover those compounds which are not offered commercially but which can be prepared from available starting materials. In general, isolation methods will be emphasized, but if certain useful compounds can be obtained by synthesis, the latter will be used. It is also planned to include techniques of general application.

Like the practice followed by *Organic Syntheses* and *Inorganic Syntheses*, the contributors have made their directions for the preparations very specific and the procedures have been checked in an independent laboratory. The 16 procedures describe the preparation of 19 compounds.

Of interest to the enzyme chemist, isolation methods are given for adenosine-triphosphate (ATP), adenosinediphosphate (ADP), and diphosphopyridine nucleotide (DPN), as well as for α -glucose-1 phosphate and for D,L-glyceraldehyde-3

phosphoric acid. In the field of the amino acids, L-serine and L-alanine are prepared from silk, with tyrosine and glycine as by-products. For their separations, three more strictly organic compounds are needed for which methods of preparation are given. A preparation of L-lysine hydrochloride from a protein hydrolyzate is given as well as the isolation of dopa from the Georgia velvet bean. Glutamine is prepared from beets and the biological aspect is emphasized when the method points out how the content of glutamine may be increased by the application of ammonium sulfate to the growing beets. In the field of protein isolations, a method for obtaining casein from milk is outlined together with two methods for isolating lysozyme from egg white.

The racemization of an amino acid through the azlactone is described for tyrosine, the resolution of the mixture is achieved through the fractionation of the brucine salts of acetyl D,L-tyrosine. The lipide field is represented by an isolation of lycopene; this involves the technique of chromatography.

A worth-while feature incorporated into this initial issue is a section under each preparation on the properties and the purity of the product. Included is a list of 58 preparations in *Organic Syntheses* which are of biochemical interest. The manual conforms to the high standards set by the sister series for organic and inorganic syntheses and the editorial board is to be commended for its efforts. Readers of this journal will no doubt find the preparative methods in this and succeeding issues of great help when they wish to study isolated biochemical systems.

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Aquametry. Application of the Karl Fischer Reagent to Quantitative Analysis Involving Water. Volume V. Chemical Analysis. J. Mitchell, Jr., and D. M. Smith. 51 illus. 143 tables. 456 pp. Interscience Publishers, Inc., New York. 1948. Price \$8.00.

This fifth volume of a series of monographs on analytical chemistry is a comprehensive treatment of the application of the Karl Fischer reagent to quantitative analyses involving water. The authors, who are both from the laboratories of E. I. duPont de Nemours Co., Inc., have coined the term "aquametry" to denote the analytical process of measurement of water; the content of this book is, however, more accurately described by the subtitle.

A short introductory chapter of 15 pages is devoted to a brief review of the various methods of determining water. The remaining 245 pages of Part I discuss fully the application of the Karl Fischer reagent to the determination of water. This discussion comprises the history, chemical background, and the various analytical techniques which include macro, micro, visual, electrometric, and other procedures. This is followed by a discussion of the application of the Karl Fischer method to the determination of water in organic and inorganic chemicals and industrial products. Of interest to cereal chemists will be the sections on moisture determination in food-stuffs, carbohydrates, and edible oils. Data are given on wheat, barley, wheat flour, oat flour, corn flakes, cottonseed, and similar products. Finally Part II, comprising 134 pages, deals with organic reactions in which water is liberated or consumed and with the determination of organic functional groups with the Karl Fischer reagent.

This book is essentially a laboratory text in which the various procedures are described in sufficient detail so that only occasional reference to original literature would be necessary. Its chief merit lies in the service which the authors have done by bringing together the voluminous literature which has appeared since the original discovery of the Karl Fischer reagent in 1935. In the opinion of the reviewer, the value of this monograph would have been enhanced had the authors allotted more space to the discussion of the basic methods of water determination, and perhaps less to the development of the less well-established applications of the Karl Fischer method.

The paper, typography, and binding are good. The book is written in a clear and logical manner, and is almost free from errors.

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FACTORS CAUSING THE CHECKING OF MACARONI¹

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ABSTRACT

The modulus of rupture, modulus of elasticity, coefficient of thermal expansion, and coefficient of moisture shrinkage of macaroni products were determined. The moisture gradients present during the drying were also measured and these data have been applied to compute the stresses present in macaroni. For commercial products the coefficient of thermal expansion was found to be 54×10^{-6} inches per inch—°C.; the coefficient of moisture shrinkage varied linearly with moisture content from 0.003 inch per inch—per cent moisture at 6% moisture to 0.0014 at 26% moisture; the average modulus of rupture of commercial spaghetti was 5,430 lb. per square inch.

The thermal stresses are small compared to the moisture stresses and to the strength of the macaroni. Plastic flow (creep) occurs at high moisture levels, relieving the stress which accompanies the formation of a moisture gradient. Reduction of the moisture gradient towards the end of drying sets up tensile stresses in the interior and compressive stresses at the outer surface which are sufficient to cause checking. Changes in relative humidity of the surroundings may produce moisture gradients due to the loss or gain of water which if sufficiently large will produce checking. The checks originate at the points of highest tensile stress.

A good macaroni has been defined as a product having the characteristics of hardness, brittleness, translucency, elasticity, and a rich amber color. The fracture should be glassy and long pieces should be sufficiently pliable to allow considerable bending before breaking.

The drying of macaroni is the most important operation in macaroni manufacture since the quality of the finished product largely depends upon the skill and judgment with which it is carried out (12). Some fundamental studies (9) on sheet macaroni dried at constant drying conditions show that macaroni exhibits a typical drying rate curve. The constant rate period ends at the critical moisture content of approximately 15% free water and is followed by the falling rate period.

Commercial drying (14) is divided into three stages, the preliminary

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drying, remoistening or sweating, and the final drying. Preliminary drying should reduce the water content from 45% (dry basis) to approximately 29–31% in 30 to 60 minutes depending upon the shape. This drying stabilizes the shape of the macaroni, prevents mold formation, and shortens the total time of drying. Remoistening or sweating consists of stopping the drying by an increase in the humidity. This allows the moisture to become uniformly distributed in the macaroni. In the final drying the moisture content of the macaroni is gradually reduced to 12.5% on the wet basis (14.3% dry basis). This period requires the longest drying time and the most careful control of the drying conditions. Numerous drying schedules are in use, some of which are described by Hoskins (11). Binnington and Geddes (7) described a procedure for experimentally drying macaroni using a constant temperature and a gradually decreasing humidity. Commercial driers are discussed by Hoskins.⁴ Improper control of drying leads either to cracks and checking or to mold formation.

The objective of the present research was to determine the factors in drying which affect the physical properties of macaroni with particular reference to checking and cracking. The coefficient of thermal expansion, the coefficient of moisture shrinkage, and the modulus of rupture were determined and the data used to compute stresses in the macaroni due to temperature and moisture gradients.

Determination of Physical Constants of Macaroni Products

Thermal Expansion. Length changes were measured by a quartz tube dilatometer patterned after that described by the American Society for Testing Materials (2). The outer tube had a length of 20 inches and an inside diameter of $\frac{1}{8}$ inch. The inner rod had a length of $11\frac{5}{8}$ inches and a diameter of $\frac{3}{16}$ inch. A Federal dial gauge graduated to 0.0002 inch per division was bolted to a bracket on the quartz tube, the stem of the dial gauge being concentric with the quartz tube.

Samples of commercial and experimentally processed macaroni were cut to a length of 3.4 inches with ends perpendicular to the axis of the tube. The length of the conditioned test specimen was measured at room temperature with a vernier micrometer to the nearest 0.001 inch. The test specimen was mounted in a dilatometer placed in a bath maintained at 30.1°C. for 10 minutes, a sufficient time for the macaroni to reach bath temperature. The reading of the dial gauge was recorded. The dilatometer was carefully transferred to a second bath^a at 3.7°C. or 67.8°C. and the dial gauge read after 10 minutes.

⁴ Private communication.

The dilatometer was returned to the first bath and the procedure repeated.

The average coefficient of linear thermal expansion was calculated by the formula

$$L = L_o[1 + \alpha(T - T_o)]$$

where α = the coefficient of linear thermal expansion inches per inch of length per °C.

$(L - L_o)$ = the average of the changes in length due to heating and cooling.

L = the length at temperature T .

L_o = the length of the test specimen at temperature T_o .

$(T - T_o)$ = the temperature difference in °C. over which the changes in length are measured.

The linear coefficients of thermal expansion which were found are presented in Table I.

TABLE I
COEFFICIENT OF THERMAL EXPANSION OF MACARONI

Source	Protein ¹	Moisture	Temperature range	Inches per inch of length per °C.
	%	%	°C.	α
Commercial	11.8	11.1	20-80	54×10^{-6}
Experimental, Stewart wheat	11.8	8.6	3.7-30.1	55×10^{-6}
Experimental, Stewart wheat	11.8	8.6	30.1-67.8	64×10^{-6}

¹ 14.0% moisture basis.

Coefficient of Moisture Shrinkage. Six pieces of commercial macaroni, protein 10.7% (14.0% moisture basis), containing 31.5% moisture (dry basis), which had been made on a conventional hydraulic press subjected to a two-hour preliminary drying with air having a 7°F. wet bulb depression, were rapidly cut with a razor blade to a length of 1.9 inches. All were marked with the light impression of a pair of dividers set at 2.530 cm. The samples, kept in a glass weighing bottle, were weighed and then placed successively in desiccators filled with sulfuric acid of the proper concentration to maintain 80, 60, 40, and 20% relative humidity. A vacuum oven was used to obtain a dry sample. The distance between the gauge marks was measured every two or three days with a Gaertner traveling microscope capable of being read to 0.0001 cm. The samples were then placed in the desiccator of lower relative humidity and the process repeated.

The coefficient of linear shrinkage due to changes in moisture

content was calculated by the formula

$$L = L_o[1 + \alpha'(W-W_o)]$$

where α' = the coefficient of linear shrinkage inches per inch of length per unit percentage decrease in moisture content (on a dry basis).

L = the length at the moisture content W .

L_o = the length at the moisture content W_o .

$(W-W_o)$ = the difference in moisture content per cent on a dry basis.

The values of α' , coefficient of linear shrinkage due to changes in moisture content for long macaroni, decreased with an increase in moisture content, having a value of 0.003 inch per inch of length per per cent change in moisture at 7% moisture, 0.0025 at 19% moisture, and 0.0017 at 26% moisture. These moisture values are all expressed on a dry basis.

Modulus of Rupture. The flexural properties of macaroni products were determined using a modification of the Flexural Test of Plastics described by the American Society for Testing Materials (1). The breaking loads for spaghetti were too small for available testing machines. A torsion balance (capacity 1 kg.) was equipped with a constant-level water-feed and receiver. The water-feed rate was adjusted to give the desired rate of loading. The water supply was cut off by a momentary contact switch on the balance which operated a manual reset relay which shut off the water supply and stopped an electric timer. A sample support rested on the other pan of the balance, above the center of which was a fixed arm for center loading of the specimen. An optical system comprised of a galvanometer lamp, a 90° prism, a lightweight mirror mounted on the central pointer of the balance, and a chart supported on the wall of the adjacent room was used to obtain deflection data during the flexure test. The deflection measuring system was calibrated by recording the deflection indicated by known displacements produced by a screw micrometer mounted in contact with the specimen plate. The loading nose and plate supports were cold-rolled steel 0.25 inch in diameter. A span of 2 inches was used for the tests on spaghetti and macaroni, while a span of 0.5 inch was used for egg noodles. Samples of commercial spaghetti made on a conventional hydraulic press were dried to between 26 and 10.9% (dry basis) in air-conditioned dryers, placed in closed containers, and tested as rapidly as possible to minimize any surface drying effects.

The modulus of rupture (flexural strength) was calculated from the load required to break the specimen, tested as a simple beam loaded at midspan, by the following formulas:

- a. For a solid cylinder of circular cross section

$$S = \frac{8PL}{\pi D^3}$$

- b. For a hollow cylinder of circular cross section

$$S = \frac{8PLD}{\pi(D^4 - d^4)}$$

- c. For a beam of rectangular cross section

$$S = \frac{3PL}{2bd^2}$$

where S = modulus of rupture, pounds per square inch
 P = breaking load, pounds
 L = distance between supports, inches
 D = outside diameter of beam tested, inches
 d = inside diameter of beam tested, inches, or
 b = width of rectangular beam tested, inches.

As shown in Fig. 1, the modulus of rupture of samples of spaghetti, protein 12.3% (14.0% moisture basis), taken at varying stages in the drying process increases as the material becomes drier. The maximum strength for these samples was 2,270 lb. per square inch for a moisture content of 7% (dry basis). It is possible that these samples were left with a high moisture gradient when the final drying was started; this would lead to large permanent stresses which would account for these lower values. Also shown in Fig. 1 is an estimated curve for commercial spaghetti using the value of 5,400 lb. per square inch at 11% moisture (dry basis).

The importance of a relatively high modulus of rupture in determining the amount of broken macaroni found on the bottom of the drying racks has been brought out by Winston and Jacobs (23). The improper control of the drying operation has much larger effects on the breakage than does the addition of lecithin. A macaroni which is full of checks must be sold as feed or if clean is sometimes reground and reprocessed.

The breaking strengths of commercial and laboratory processed macaroni have been presented by Binnington and Geddes (7) in a form which permits the calculation of the modulus of rupture. Commercial macaroni had a modulus of rupture lying between 6,100 and 7,300 lb. per square inch. Laboratory-processed material had a modulus of rupture lying between 3,600 and 5,200 lb. per square inch.

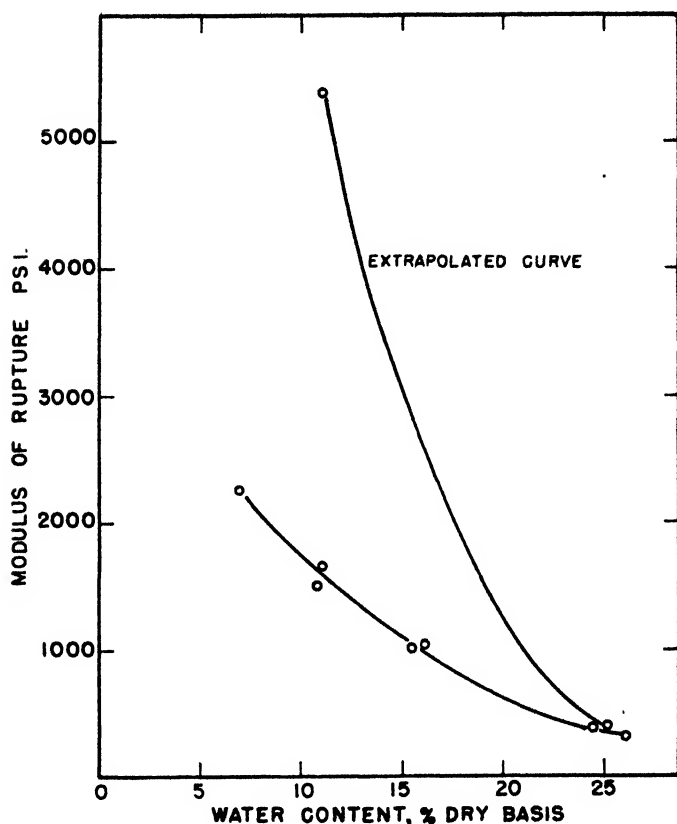


Fig. 1. Modulus of rupture of spaghetti as a function of water content. The extrapolated curve is an estimated one for commercial spaghetti using a value of 5,400 lb. per square inch for the modulus of rupture at 11% moisture (dry basis).

The modulus of rupture has been determined for four samples of commercial spaghetti, protein 11.5% (14.0% on moisture basis), having a moisture content of 11% (dry basis). The average of the 10 readings from each sample was used to compute the modulus of rupture presented in Table II.

TABLE II
MODULUS OF RUPTURE OF COMMERCIAL SPAGHETTI

Diameter in.	Modulus of rupture lb./sq. in.
0.064	5,120
0.073	5,770
0.085	5,500
0.089	5,340
Average	5,430

Binnington, Johansson, and Geddes (8) have reported the relation between protein content of wheat and the breaking strength of macaroni. Their data reported in arbitrary units have been converted for purposes of comparison into physical units, modulus of rupture in pounds per square inch, in Table III. It was necessary to assume that the distance between supports in their flexural test was 1 inch.

Winston and Jacobs (23) have reported the breaking strength of commercially dried spaghetti processed with and without added lecithin. The sample was supported on glass rods 6 inches apart and weights were added at the center until the breaking point of the spaghetti was reached.

TABLE III
EFFECT OF PROTEIN LEVEL ON THE MODULUS OF RUPTURE

Protein content ¹	Mean breaking strength	Modulus of rupture
%	Units	lb./sq. in.
10.5	153.0	3,978
11.3	166.2	4,321
12.3	169.5	4,407
13.2	190.4	4,950
13.9	196.8	5,117

¹ 14.0% moisture basis.

Assuming that the diameter of the spaghetti was 0.080 inch, the modulus of rupture, computed from their data is 4,333 lb. for the control and 4,240 lb. for the spaghetti to which 0.5% lecithin had been added. The addition of 0.5% lecithin does not have an appreciable influence upon the breaking strength of spaghetti in comparison with the larger effects attributable to drying conditions. Winston and Jacobs provide data showing that the use of lecithin increases the amount of broken pieces from 0.28% for the control to 0.34% with the added lecithin.

Young's Modulus of Elasticity. Modulus of elasticity may be defined as the ratio of the internal stress to the strain and is a number characteristic of the material at a given moisture content. The procedure already described for determining the modulus of rupture was followed in measuring the modulus of elasticity. The deflections were measured at 5-second intervals during the loading of the sample.

The modulus of elasticity (Young's Modulus) of spaghetti determined as a simple beam loaded at midspan was calculated from the theory of flexure using the elastic curve of a beam, with the formula

$$E_b = \frac{4L^3P}{3\pi D^4} \\ = \frac{4L^3(P70 - P20)}{3\pi D^4(\Delta70 - \Delta20)}$$

where E_b = modulus of elasticity
 L = distance between supports, inches
 P = load, pounds
 Δ = deflection at center, inches
 P_{70} , 70 = load and deflection at 70% of maximum load
 P_{20} , 20 = load and deflection at 20% of maximum load
 D = diameter of spaghetti, inches.

The results are presented in Fig. 2 as modulus of elasticity versus the moisture content expressed on a dry basis.

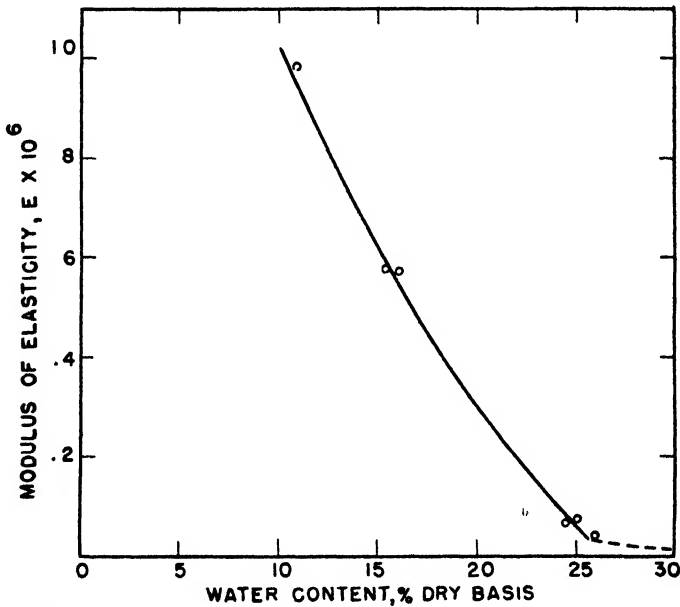


Fig. 2. Modulus of elasticity of spaghetti as a function of water content.

The modulus of elasticity rises rapidly as the moisture content is decreased below 26% and since the equipment used was not suitable for wetter material, data on modulus of elasticity of flour dough reported in the literature (16, 17, 18) have been used to extrapolate the curve to 30% water (dry basis).

Moisture Gradients in Drying

A sample of macaroni dough, protein 11.9% (14.0% moisture basis), was rolled to a thickness of 0.075 inch. Five layers were placed in an aluminum moisture dish and drying proceeded from only one face. The sample was placed in the experimental dryer described

by Ritchell *et al.* (15) and dried for 4 hours. The dry bulb was held at 35°C., relative humidity at 72%, and the air velocity at 640 feet per minute. Upon removal from the dryer, the outer layer was split into two portions, placed in aluminum moisture dishes, and analyzed for moisture by the vacuum oven method at 99°–100°C. The rate of drying below the surface was very slow. The data were plotted and from the curve it could be estimated that the level at which there had been no moisture change was in the top layer.

A sample of elbow macaroni from a Buhler Continuous press, protein 11.9% (14.0% on moisture basis), was rolled to a thickness of 0.014 inch. Seven layers were made into a sandwich and placed in the same dryer for 2 hours at 95.4°F. with a relative humidity of 70.5% and an air velocity of 670 feet per minute. The equilibrium moisture corresponding to this humidity is 16% (dry basis). The moisture content dropped from 46% to an average of 28% in 2 hours. The distribution of water obtained from the analysis of the delaminated sandwich is presented in Fig. 3. A difference of 18% existed between the center and the outside surface. The conditions of this test were similar to those which have been used in several commercial plants. In the rest period, "sweating," the surface would be expected to rise to some point between 26 and 30% either by addition of water from the air or by further diffusion of water from the center toward the surface. Plant tests have shown that a truck containing 175 lb. of wet macaroni in a period of rest often gains up to 2% water on a dry basis. This would indicate that water is added to the surface in addition to a diffusion of water from the interior.

A sample of macaroni dough handled in a manner similar to the previous tests was dried for 8 hours under the following conditions: dry bulb, 90.8°F.; wet bulb difference, 25.7°F.; relative humidity, 23%; equilibrium moisture content, 7.7% (dry basis); air velocity, 670 feet per minute. At the end of drying a difference of 11% in moisture content existed between the center and the surface. The conditions of this test are far more severe than can be tolerated in commercial practice if the product is to be in one piece free from large cracks. This test indicates that a commercial spaghetti of 0.062 inch radius could drop from the moisture content it contains when pressed (44–46%) to 19% in 8 hours. However, the gradient in the early stages of drying must have exceeded 25% moisture (dry basis) and this is more than the material can withstand without checking.

Samples of elbow macaroni, protein 11.9% (14.0% moisture), were obtained from a Buhler Continuous press in a local plant. The dough which passed through the die has been assumed to have a diffusivity similar to the extruded material when rolled out between steel rollers.

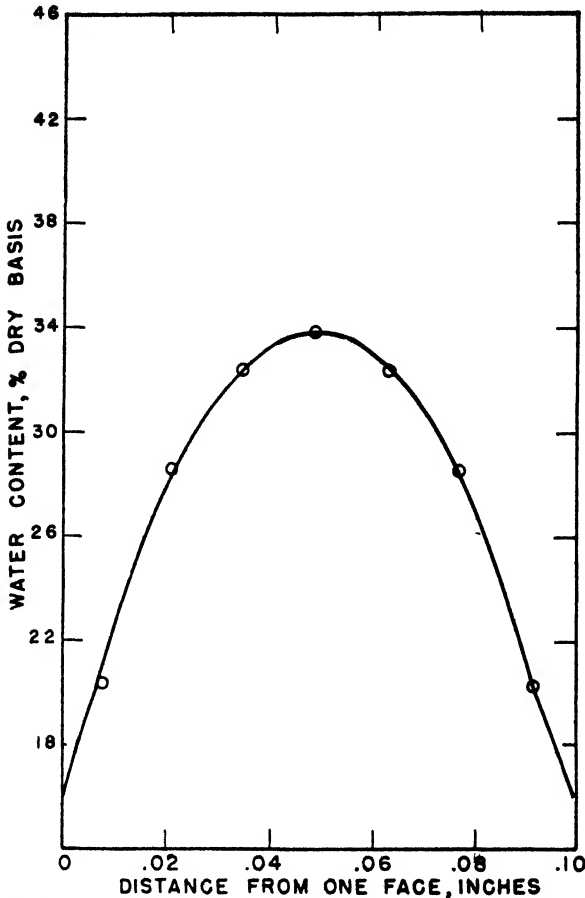


Fig. 3. Moisture gradients in preliminary drying. The data were obtained by drying a sandwich of seven sheets, each 0.014 inch thick, for 2 hours at 95.4°F. with a relative humidity of 70.5% and an air velocity of 670 feet per minute, and determining the moisture content in the various layers of the delaminated sandwich.

The dough was rolled out to a thickness of 0.02 to 0.03 inch and folded to make a sandwich of seven layers, approximately 2 by 5 inches. The four edges were pressed together by a $\frac{1}{8}$ inch wide straightedge to prevent delamination during drying. Seven similar samples were placed in a horizontal position supported at the edges by a thin wire framework 2 inches above the floor of a controlled humidity dryer described by Ritchell, Piret, and Mann (15). The average air velocity used in this drying experiment was 637 feet per minute; the dry bulb temperature was 35°C. The humidity was varied during the experiment, to approximate a commercial schedule for a 30-hour drying period. At stated periods of time one of the seven similar samples

was removed, the edges trimmed back to eliminate those portions where drying was occurring also through the edges, and carefully delaminated with a razor. Each layer was placed in an aluminum moisture dish with a tight-fitting cover and the moisture content was determined by drying in a vacuum oven at 100°C. for 48 hours without grinding the sample. An attempt was made to simulate the commercial methods by 1 hour of rapid drying at a low relative humidity (80%) followed by a rest period of 1 hour in air of high relative humidity (93%) with the fan turned off. The fan was then turned on and the drying was continued.

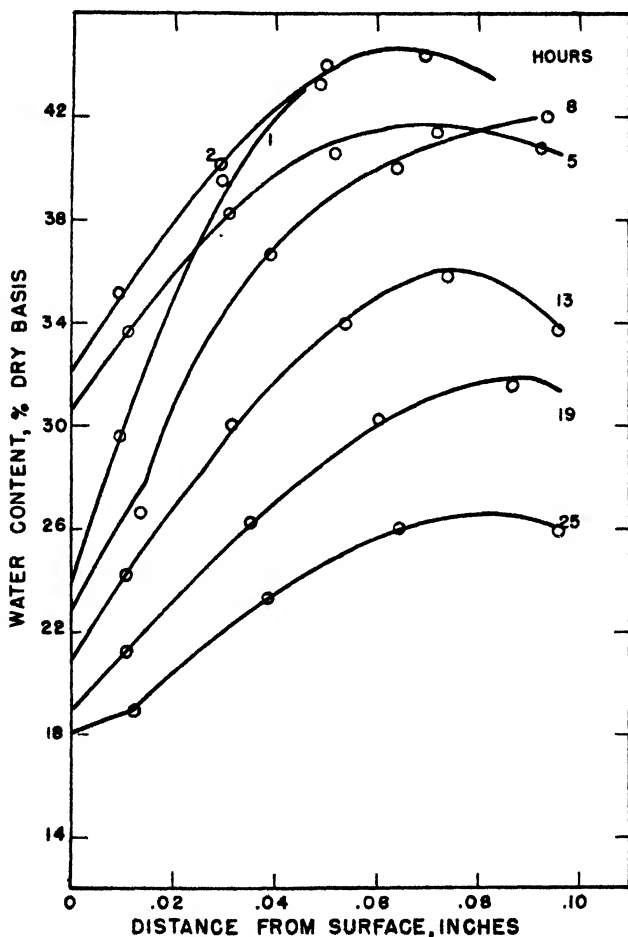


Fig. 4. Moisture gradients in drying macaroni at 35°C. for different times. The data were obtained by drying seven sheets of macaroni dough, each 0.02 to 0.03 inch thick, made into sandwiches at 35°C., with the relative humidity varied to approximate a commercial 30-hour drying schedule. At the stated time intervals one sandwich was removed, the layers separated, and their moisture contents determined.

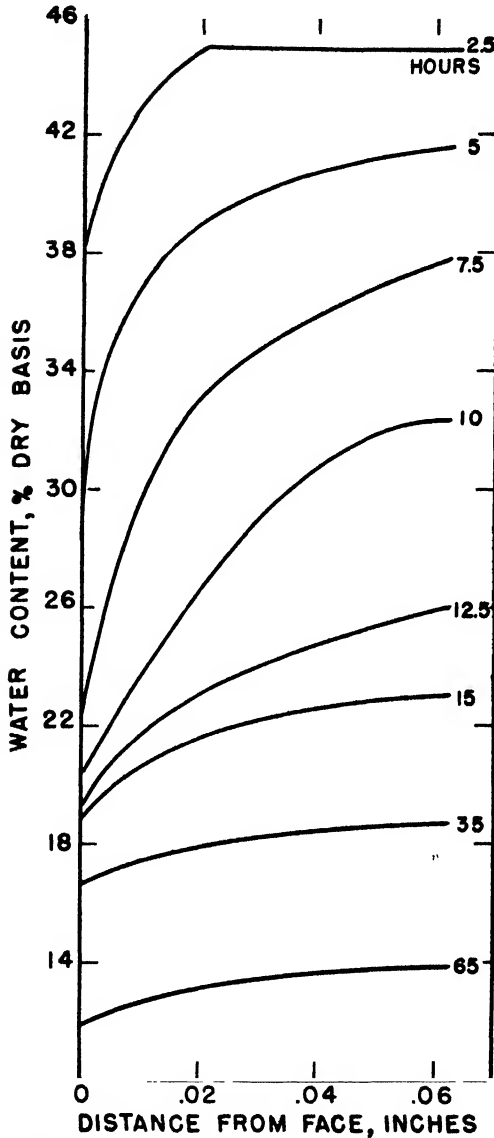


Fig. 5. Moisture gradients in drying macaroni for different times. The curves are based on data presented by Binnington and Geddes (7) for the laboratory drying of macaroni.

The moisture content of the macaroni as a function of distance below the drying surface is plotted in Fig. 4 for drying times of 1, 2, 5, 8, 13, 19, and 25 hours. The preliminary drying for 1 hour at a relative humidity of 80% established a moisture difference of 22% (dry basis) between the surface and the center of the slab. The

effect of the sweating was to add water to the surface, raising the surface moisture content from 21 to 30%; this had the over-all effect of reducing the moisture gradient in the slab from 22 to 13% (dry basis). The moisture content of the surface was gradually decreased by lowering the relative humidity and this resulted in a gradient between the center and the outside of 13% after 13 hours. After 25 hours the moisture gradient was still 8.5%.

The moisture distribution has been approximated in Fig. 5 from the data presented by Binnington and Geddes (7) for the laboratory drying of macaroni. The gradients in the early stages of drying are large in this test because of the short preliminary drying which was given the macaroni.

Discussion of Factors Causing the Checking of Macaroni

Semolina when mixed with water first forms a crumbly mass which is then kneaded into a dough and extruded by hydraulic or continuous-type presses. The dough in this form is plastic but it gradually loses this property during drying and behaves more nearly like a brittle material, that is, a material which is weakest in tension. Macaroni in the various stages of its drying is initially ductile (i.e., capable of being permanently pulled out), then it is both ductile and elastic in the region where viscous flow relieves stress, and is finally brittle at lower moisture contents. There is no sharp dividing line in which the properties suddenly change from one type to another. The location of a region over which viscous flow can occur is not sharply defined at this time although many plant operators find that one-third of the water in the dough can be removed rapidly and still produce a satisfactory product. The modulus of elasticity curve may be extrapolated to zero at a moisture content between 26 and 27% (dry basis). However, if the data from the literature for a bread dough are used to extend this curve, a sharp change in the slope occurs in this same moisture range.

Large samples of elbow macaroni taken from a commercial short-goods dryer were stored in closed cans for a period of 1.0 and 1.5 hours. The samples were examined by a manufacturer of macaroni drying equipment and by the authors. The lower limit for satisfactory re-moistening was considered to be between 26.5% for an airtight remoistening chamber and 30% (dry basis) for a remoistening chamber in which there was a lower relative humidity. The amount of bound water computed from data published by Swanson (19) was found to be 28.2% (dry basis). Vail and Bailey (22) found that the bound water was 28.6% (dry basis) at freezing temperatures, while Baker, Parker, and Mize (5) calculated the bound water to be 27.3% (dry basis).

The data indicate that the limiting moisture content permitting viscous flow lies between 26 and 30% (dry basis). This must be further qualified by a consideration of the strength of brittle solids. At low temperatures a point is often reached called the brittle point. This probably represents the temperature at which both elastic modulus and plastic viscosity become so high that sufficient deformation cannot take place rapidly enough to prevent the stresses imposed in a short-time test from exceeding the strength of the material.

A brittle material shows elastic properties including not only the instantaneous response but also lesser known reactions, such as primary creep, elastic after-working, and delayed elastic effect. The strength of a brittle material depends on such factors as time, chemical environment, and prestressing or permanent stresses which result from the methods of formation and drying. Recent theory applied to brittle materials emphasizes that various molecular or atomic mechanisms are the essential factors which determine the time for fracture. Since the elementary process of rupture consists of the irreversible separation of the atoms or molecules, no other external force except tension can bring about this separation. Failure occurs at the point having the largest tensile stress. Prestressing of the surface under compression increases the tensile strength by making surface flaws less important. Poncelet (13) states that it is unnecessary to resort to any hypothesis of the preexistence of flaws to explain the behavior of homogenous brittle solids under stress; the application of mechanics and elasticity to the particulate structure of matter and of the electrostatic character of the particle appears to suffice. A fracture in a brittle solid begins at or near the most positive principal stress, and once started tends to propagate further. The condition of the outside surface, the atmosphere in direct contact with the solid, and the duration of the stress all have an effect on the value of the strength found for a brittle solid. The tensile strength of identical solids always shows a scattering of values instead of identical reproducible values which are characteristic of common ductile materials. Binnington, Johansson, and Geddes (8) found a large scattering of their results for the flexural strength of macaroni; which was not due to the differences in dimensions. The theory of the failure of brittle solids provides an explanation of their observation.

The stresses that originate in a tubular strand of macaroni or spaghetti which is subjected to a moisture gradient may be treated by the equations developed for stresses which originate from a change in tempering (6, 20, 21). The moisture gradient is quite similar in shape to the thermal gradient that has been used in the derivation of the

thermal stress equations. Moisture stresses may arise from one of the following causes: (1) an increase in moisture content at the surface, the moisture content of the sample having been uniform; (2) a decrease in moisture content at the surface, the moisture content of the sample having been uniform; and (3) the removal of water from a wet dough.

Increase in Moisture Content of the Surface. A solid tube or cylinder that has a uniform moisture distribution placed in an air stream of the same temperature, but whose relative humidity is greater than that corresponding to the equilibrium value for the moisture in the macaroni or spaghetti, will pick up water from the air. As water is added to the surface of the macaroni the outside of the tube tends to expand more than the inner layers. The outside of the cylinder is not free to expand in the normal manner and is therefore under compression; at the same time the inner layers will be stretched by the outer layers and will be in tension. The longitudinal and tangential stresses change continuously from compression at the surface to tension in the interior and necessarily pass through an intermediate zone of zero stress. The stresses formed are tabulated below:

DIRECTION OF STRESS	LOCATION	TYPE
Tangential stress	Inside radius	Tension
Tangential stress	Outside radius	Compression
Longitudinal stress	Inside radius	Tension
Longitudinal stress	Outside radius	Compression
Radial stress	All radii	Tension

As long as the outside layer is in compression, the macaroni will be stronger and will be less likely to check on the outside surface, but still may fail by checking in the interior due to the tensile stress at the inside radius. If water is added until the moisture distribution is again uniform at the new level corresponding to the relative humidity of the air surrounding the macaroni, the stresses caused by this moisture gradient will have disappeared with that gradient. These stresses may be called temporary since they continue only as long as the moisture gradient is maintained.

Decrease in Moisture Content of the Surface. A solid tube or cylinder which has a uniform moisture distribution when placed in an air stream of the same temperature but of a lower relative humidity than corresponds to the equilibrium value for the moisture in the macaroni will lose water to the air. This establishes a moisture gradient in the sample. Temporary stresses will be developed as shown in the table below:

DIRECTION OF STRESS	LOCATION	TYPE
Tangential stress	Inside radius	Compression
Tangential stress	Outside radius	Tension
Longitudinal stress	Inside radius	Compression
Longitudinal stress	Outside radius	Tension
Radial stress	All radii	Compression

The macaroni will be weakest when the outside surface is in tension and failure will be most likely to occur at the outer surface. When equilibrium is reached at the lower moisture level corresponding to the new relative humidity, the temporary stresses caused by the moisture gradient will have disappeared.

Removal of Water from a Wet Dough. The removal of water from macaroni causes the formation of moisture gradients as shown in Figs. 3, 4, and 5. The use of controlled relative humidity schedules or periods of alternate drying and resting have been used to minimize the magnitude of these gradients.

The stresses established by drying may be considered under the two following cases: (1) Macaroni with a uniform moisture gradient exists in the stress-free condition when it passes the lower limit of moisture content where stresses can be relieved by viscous flow; (2) A moisture gradient exists within the stress-free macaroni when it passes the lower limit of moisture content where stresses can be relieved by viscous flow.

STRESS-FREE MACARONI, NO MOISTURE GRADIENT. Drying will put the macaroni under a temporary stress and so long as the macaroni is not checked at any point due to an excessive gradient, the strength will be regained when the moisture distribution again becomes uniform. There will be no permanent stresses in this macaroni. The strength of this macaroni would be greater if the surface were under a slight compression, for this would minimize the effect of the surface irregularities as stress concentrators.

STRESS-FREE MACARONI, MOISTURE GRADIENT PRESENT. In this case a moisture gradient exists in a material that is free from stress such as might well exist in macaroni where plastic flow has occurred to release the stresses formed in the early stages of drying. The removal of this gradient will cause stresses opposite in sign but equal in magnitude to the stresses which would be caused by the same moisture gradient in a sample initially free of stress. The removal of such moisture gradients will cause stresses the same as shown above.

These stresses are called permanent stresses and can be removed permanently only by an annealing process. It has been possible to remove permanent stresses in the cases of certain thermoplastics by the addition of radiant heat to raise the temperature of the plastic

above the point at which internal stresses are relieved. This is not considered practical for macaroni for several reasons. Macaroni tends to decompose before any softening point is reached, color would be injured at higher temperatures, and there would be the cost and difficulty of cooling the macaroni without checking it due to excessive changes in moisture content at the surface.

The customary preliminary drying followed by a remoistening or sweating to obtain a uniform moisture distribution tends to produce a stress-free macaroni with a moisture gradient. A portion of the stresses are released by viscous flow during the remoistening period. If moisture equilibrium was not obtained and drying was started again, it would be possible to leave the region where internal stresses can be relieved with a large moisture gradient but with relatively stress-free macaroni. In this case, no matter how much care is given in drying, the final product will be weak due to the permanent stresses and may even be checked on the inside as the moisture gradient is reduced at the end of the drying period.

A like case would result from a short preliminary drying with remoistening at a high moisture level, followed by a resumption of drying establishing a new moisture gradient by the time the material has reached the lower limit for plastic flow. Where this moisture gradient is large the macaroni may fail by tension at the inner surface upon the removal of the moisture gradient at the end of drying. This type of failure is one of the most puzzling to commercial operators, for the macaroni looks and feels strong as long as the drying is in progress. Soon after the fans are stopped the cracking starts.

It is not likely that there would be a sudden change in properties above a certain moisture content. It is more likely that when there is some free water present (above about 26% on a dry basis), some viscous flow can occur. This releases a portion of the stresses permitting a macaroni with a moisture gradient to exist in a stress condition less than would be shown by computation. Thus one would not expect to attain a stress-free condition when dry. A strong product would result at the end of drying if one passed through this region with a small moisture gradient. This macaroni is, then, more susceptible to surface cracking in the earlier stages of drying than when the permanent stresses place the outer surface in compression.

The equations of Barker for the thermal stresses in a long tube of circular cross section may be modified for the computation of the maximum tangential stress due to the formation of a moisture gradient between the inner and outer surface of the tube. The modified equations in which moisture, W , is substituted for temperature, T , and the moisture coefficient of shrinkage, α' , is substituted for the

thermal coefficient of expansion, a , are as follows:

$$P_r = \frac{(W_1 - W_2) \frac{1}{\gamma} E \alpha}{2 \left(\frac{1}{\gamma} - 1 \right) (r_2^2 - r_1^2) \ln \frac{r_1}{r_2}} \left[\frac{r_1^2 r_2^2}{r^2} \ln \frac{r_1}{r_2} \right. \\ \left. + r_2^2 \ln r_2 - r_1^2 \ln r_1 - (r_2^2 - r_1^2) \ln r \right]$$

$$P_\theta = \frac{(W_1 - W_2) \frac{1}{\gamma} E \alpha}{2 \left(\frac{1}{\gamma} - 1 \right) (r_2^2 - r_1^2) \ln \frac{r_1}{r_2}} \left[\frac{r_1^2 r_2^2}{r^2} \ln \frac{r_2}{r_1} \right. \\ \left. - (r_2^2 - r_1^2) (\ln r + 1) + r_2^2 \ln r_2 - r_1^2 \ln r_1 \right]$$

$$P_z = \frac{(W_1 - W_2) \frac{1}{\gamma} E \alpha}{2 \left(\frac{1}{\gamma} - 1 \right) (r_2^2 - r_1^2) \ln \frac{r_1}{r_2}} \left[2 r_2^2 \ln \frac{r_2}{r} \right. \\ \left. - r_1^2 \ln \frac{r_1}{r} - (r_2^2 - r_1^2) \right]$$

where $W_1 - W_2$ = difference in water content between the inside and outside of the tube, per cent on a dry basis

T = temperature of the tube, °C.

r = radius of the tube, inches

γ = Poisson's ratio for the material

E = modulus of elasticity, Young's

a' = moisture coefficient of shrinkage of the material

P_θ = tangential tensile stress

P_r = radial tensile stress

P_z = longitudinal tensile stress

Suffixes 1 and 2 refer to the inside and outside of the tube, respectively.

A solution for a typical case where the ratio of r_2/r_1 is 1.7 gives the maximum tangential stress produced by the formation of a moisture gradient of 1% to be 1,800 lb. per square inch.

The theoretical moisture gradient which will not cause checking has been computed for the two modulus of rupture curves of Fig. 1. Using the experimental data, the macaroni will withstand a moisture gradient of from 0.75 to 1.5% without fracture, depending on the ratio of r_2/r_1 and the moisture content. From the extrapolated data on the commercial product, the gradient varies from 1.5 to 2.5%. A thin-walled product can withstand a greater stress than can a thick-

walled product. These results merely indicate trends since the failure in brittle solids depends on many factors, including the magnitude of the initial stress distribution. It is entirely possible that the allowable tensile stress of stress-free macaroni may be several times greater than has been determined.

The effect of temperature alone can be calculated for a hypothetical case by assuming that macaroni containing 12% moisture (dry basis) at 90°F. is suddenly placed in a current of moving air at 70°F. with a relative humidity such that no changes occur in the surface moisture content. The maximum tangential stress calculated for these conditions is 19 lb. per square inch which is a very small value in comparison with the allowable value of 2,000 to 5,000 lb. per square inch for the modulus of rupture.

Observation of relative humidities on various floors of commercial plants has shown that relative humidity variations from 65 to 50% are not uncommon. Variations from 70 to 30% have been measured in the winter on numerous occasions.

Macaroni in equilibrium with air of 65% relative humidity would have a moisture content of 14.9% (dry basis), while after attaining equilibrium in air of 50% relative humidity, the moisture content would be 12.3% so that the moisture gradient between the surface and the interior would be 2.6%. The maximum tangential stress due to a difference of 1% was calculated to be 1,800 lb. per square inch. For a difference of 2.6% the maximum tangential stress would be 4,700 lb. per square inch. This would not cause checking of a strong macaroni.

LeClerc (12) has stated that macaroni should not be subjected to sudden changes in temperature as this may cause the product to warp. A study of the relative magnitude of the stresses due to temperature and relative humidity changes shows clearly that it is the moisture content of the air and not the temperature that is responsible for the checking of macaroni products. Probably the origin of this line of thought in the industry lies in the fact that a plant having widely different temperatures will also have large differences in the relative humidity.

A second cause of checking is the disappearance of the moisture gradient that existed in the macaroni when it was in the plastic state in a stress-free condition.

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EFFECT OF HEAT TREATMENT ON THE SULFHYDRYL GROUPS OF MILK SERUM PROTEINS¹

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ABSTRACT

When milk serum protein sols containing 5.0 to 6.0 g. of protein per liter in phosphate buffer at pH 6.6 were heat-treated under nitrogen, dialyzed, and stored in air, the titratable —SH groups expressed as cysteine and determined by the o-iodosobenzoate procedure decreased from 0.87% for the control to 0.68, 0.51, and 0.42% for sols maintained at 70°, 75°, and 80°C. respectively for 30 minutes. These decreases could not be accounted for by the small quantities of volatile sulfur which were evolved nor by any change in the cysteine or methionine values. Failure to detect cystine in hydrolyzates of either unheated or heated samples indicates that the lower —SH titer of the heat-treated samples was due to aggregation of the protein molecules rather than to oxidation of sulfhydryl to disulfide linkages.

When milk serum protein sols were heat-treated in air and nitrogen respectively and titrated immediately upon cooling, a decrease in —SH groups available to o-iodosobenzoate only occurred in air. Both thiamine disulfide and ferricyanide gave very low titers for unheated milk serum protein, the —SH values expressed as cysteine being 0.02 and 0.09% respectively as compared with 0.63% by titration with o-iodosobenzoate. However, heat treatment increased the groups available to these reagents.

The possibilities of heat-induced interactions between the several electrophoretically different components of the milk serum and of the protein sulfhydryls with lactose are discussed.

Since sulfhydryl compounds cause softening of dough and depression of loaf volume, the decrease in titratable —SH groups as measured by titration with o-iodosobenzoate serves to explain the improvement in the baking value of nonfat milk solids that can be effected by the proper heat-treatment of separated milk before drying.

It is well known that the detrimental effect of nonfat dry milk solids on the consistency of dough and the loaf volume of bread can be overcome by heating the separated milk before drying (11, 12, 23, 28). Furthermore, the serum protein fraction of the milk has been shown to be responsible for both the dough softening (23, 30) and the loaf volume depression (15, 23).

The fundamental reason for the dough-softening action of the milk serum proteins has not been elucidated satisfactorily. However, the

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similarity between this phenomenon and the dough-softening effect of reducing substances (see Shen and Geddes, 27) would lead one to suspect that such powerful reducing groups in the protein as the sulfhydryls might be involved. This suggestion actually was made by Stamberg and Bailey (30), who found that while fresh skimmilk exhibited a polarographic wave characteristic of sulfhydryl compounds, such a wave could not be demonstrated with heated skimmilk. At first glance, this result appears to be contradictory to the generally accepted idea that denaturation of proteins by heat or other means liberates sulfhydryl groups (1, 26). It also seems contrary to the established fact that heat-treatment of milk causes the appearance of a positive nitroprusside test (13, 19) and of substances that reduce thiamine disulfide (14). Stamberg and Bailey (30) suggested that this difficulty might be resolved by considering that heat causes a reorganization of the protein micelles in such a way that the over-all activity of the sulfhydryl groups is decreased, while at the same time, some of the —SH groups are broken away to form free hydrogen sulfide.

Heat-treatment of milk not only produces internal rearrangements of the serum proteins, but also liberates a certain amount of volatile sulfur-containing compounds (Townley and Gould, 31, 32, 33). Such a loss of volatile sulfides must be taken into consideration in studying the effect of heat on the sulfur distribution in milk.

The present research was undertaken to examine the changes produced by heat-treating protein sols in the activity of the —SH groups and in the quantities of sulfur present as volatile sulfur, cysteine, cystine, and methionine. In the main series of experiments, the serum protein sols were heat-treated in nitrogen and the —SH groups determined by titration with o-iodosobenzoate. Supplementary studies were made in which the sols were heated in the presence and absence of air and the —SH groups determined by each of three methods (thiamine disulfide, ferricyanide, and o-iodosobenzoate).

Materials and Methods

The major portion of this investigation deals with a single lot of milk serum protein. Sols of this protein at pH 6.6 were subjected to selected heat-treatments. The volatile sulfides evolved in the process were collected and determined, and the sols were analyzed for titratable sulfhydryl groups. Finally, the sols were dried from the frozen state under vacuum and the dried material was analyzed for cysteine, cystine, methionine, and total sulfur.

Preparation of Milk Serum Proteins. The casein was precipitated from fresh raw, separated milk at 25°C. by the acetate method described in a previous paper (23). The resulting serum was divided

into four portions each of which was dialyzed in Visking sausage casings against running tap water at 13°C. for 42 hours and then against distilled water for 6 hours. A minute amount of protein, precipitated during the dialysis, was removed by filtration. Phosphate buffer was added to each portion to yield a pH of 6.6 and an ionic strength of 0.1 in the final solution. The protein concentrations of these sols were 5 to 6 g. per liter.

Comparisons of the thiamine disulfide, ferricyanide, and o-iodosobenzoate methods for determining sulfhydryl groups were made on another lot of milk serum protein prepared in the same way.

Heat-Treatment and Estimation of Volatile Sulfides. The method employed to heat the serum protein sols was essentially that used by Townley and Gould (31) to heat milk. The apparatus consisted of a 2-liter flask immersed in a water bath and fitted with a condenser, which in turn was connected to a volatile sulfide trap containing 150 ml. of 0.5% zinc acetate in 0.4% sodium hydroxide.

One liter of each of the four sols of serum protein in buffer was successively placed in this apparatus. In each case, the solution, taken from the cold room at 3°C., was brought to 25°C. before it was introduced into the flask. The system was closed and a stream of nitrogen, freed from oxygen and hydrogen sulfide by passing through alkaline pyrogallol and silver nitrate respectively, was bubbled through it. The first lot was maintained at 25°C. for 60 minutes, nitrogen being bubbled through it during 30 minutes and a vacuum drawn with an aspirator during the last 30 minutes. The other three solutions were heated to the respective temperatures of 70°, 75°, or 80°C. in 24 to 28 minutes, maintained at this temperature for 30 minutes, and cooled to 25°C. in 30 minutes. The nitrogen stream was bubbled through the system until the end of the heating period; during the cooling period the nitrogen was stopped and a vacuum was drawn to remove any last traces of volatile sulfides. Sulfides were estimated in the contents of the sulfide collector by the colorimetric method described by Townley and Gould (31). In this method methylene blue is produced from p-aminodimethylaniline, ferric chloride, and the volatile sulfides of the sample. The intensity of the blue color was determined after 1 hour with a Coleman spectrophotometer, employing a wave length setting of 660 m μ .

Titration of Sulfhydryl Groups. After heat-treatment, the milk serum protein sols were dialyzed for 6 hours against tap water and 6 hours against distilled water to remove the buffer. Their protein concentrations changed slightly because of osmosis and consequently were redetermined.

The sulfhydryl groups of these sols were titrated with o-iodoso-

benzoate by the method of Hellerman *et al.* (17, 18). This method depends upon the oxidation at pH 7.0 of the sulfhydryl group to disulfide by o-iodosobenzoate. The excess o-iodosobenzoate is titrated iodimetrically with thiosulfate. It was possible by this method to account for 99% of the cysteine in a 0.100 *N* solution. Titration of egg albumin, prepared by the method of Kekwick and Cannan (22) in 3 *M* guanidine hydrochloride, yielded a value equivalent to 1.30% cysteine, which is in close agreement with the value of 1.29% reported by Hellerman *et al.* (17) for titrations of similar preparations in 7 *M* guanidine hydrochloride.

The titrations of milk serum proteins with the o-iodosobenzoate method were performed as follows: To 10 ml. of the protein solution 5 ml. of phosphate buffer (pH 7.0) and 1 ml. of 0.02 *N* o-iodosobenzoate solution were added. The mixture was shaken for 2 minutes. During the third minute 5 ml. of water, 1.8 ml. of 1 *N* hydrochloric acid, and 2 ml. of starch indicator were added to 0.3 g. of potassium iodide, previously weighed out in a separate flask. At the end of the third minute, the contents of the second flask were added to those of the first and the iodine liberated was titrated with 0.01 *N* sodium thiosulfate using a burette with 0.01 ml. graduations. A blank in which 10 ml. of water was substituted for the 10 ml. of protein solution was also run.

The determinations of sulfhydryl with ferricyanide and thiamine disulfide were made by the methods described by Crowe *et al.* (9) and Harland and Ashworth (14) respectively.

Estimation of Cysteine and Cystine. For the determination of cysteine and cystine 0.3 g. of the dry protein preparations were hydrolyzed for 15 hours at 110°–115°C. with 30 ml. of 6 *N* hydrochloric acid under nitrogen and analyzed by the colorimetric method of Lugg (24, 25) as modified by Kassell and Brand (20) which employs phospho-18-tungstic acid as the oxidant. Some brown humin formed during the hydrolysis. The insoluble portion of this humin was removed by filtration through a sintered glass crucible, and while some soluble humin remained it did not interfere appreciably with the estimation of the blue color of reduced phospho-18-tungstic acid because of the high dilution involved. The absorption of the blue-colored solution was measured with a Coleman spectrophotometer at 670 $m\mu$., the concentration of amino acid being read from a standard curve. In the absence of sulfite the analysis determines cysteine alone. The addition of sulfite causes reduction of cystine to cysteine and consequently both cystine and cysteine are determined if sulfite is added.

In tests on separate solutions of the pure amino acids, recoveries of 95% of the cysteine and 94% of the cystine were realized. When a

solution containing both was analyzed, 92% of the cystine and 99% of the cysteine were recovered. Application of the method to a sample of commercially prepared lactalbumin (S.M.A. Corp., Chagrin Falls, Ohio) yielded 2.80% cystine and 0.21% cysteine, which are in reasonable agreement with the respective values of 2.80% and 0.28% reported by Kassell and Brand (21) for similar material.

Estimation of Methionine. Methionine was determined in the dry proteins by the method of Baernstein (2, 3, 4) which involves hydrolysis of the protein with hydroiodic acid whereupon methyl iodide is formed from the methionine. The gaseous methyl iodide is collected in a bromine solution. Iodate is formed which is titrated with thiosulfate. Samples weighing about 0.5 g. were hydrolyzed with 10 ml. of redistilled hydroiodic acid at 130°–140°C. for 8 hours. Nitrogen was bubbled through the sample during hydrolysis. The evolved gas was washed by passing through a solution of 20% cadmium chloride saturated with respect to mercuric chloride and the methyl iodide was caught in a solution of bromine in acetic acid. At the end of the hydrolysis the excess bromine was reduced with formic acid and an aliquot of the solution titrated iodimetrically with thiosulfate.

A recovery of 93% of pure methionine from solution was obtained with this method.

Determination of Total Sulfur. Total sulfur was determined by oxidizing samples of about 0.25 g. of the dry protein in the Parr peroxide bomb, dissolving the fused mass, and precipitating and weighing the sulfur as barium sulfate.

Results and Discussion

The results of these analyses are given in Table I on an amino acid basis and in Table II on the basis of sulfur distribution.

TABLE I
TITRATABLE SULFHYDRYL GROUPS AND SULFUR-CONTAINING AMINO ACIDS
IN MILK SERUM PROTEINS AS AFFECTED BY HEAT-TREATMENT

Heat-treatment ¹	Titratable ² —SH groups (as cysteine)	Cystine ³	Cystine ³	Methionine ⁴
°C.	%	%	%	%
Control	0.87	2.9	0.0	3.2
70	0.68	—	—	—
75	0.51	—	—	—
80	0.42	3.0	0.0	3.1

¹ The heat-treated samples were raised to the indicated temperatures in 24 to 28 minutes, held at the specified temperature for 30 minutes, and cooled to 25°C. in 30 minutes.

² Mean of three determinations by the o-iodosobenzoate method.

³ Mean of six determinations, three on one weighed sample and three on another.

⁴ Mean of three determinations on the hydrolyzate from one weighed sample.

TABLE II
SULFUR DISTRIBUTION OF UNDENATURED AND HEAT-TREATED
MILK SERUM PROTEINS

Heat-treatment ¹	Volatile sulfur ²	Cysteine sulfur ³	Methionine sulfur ⁴	Total sulfur ⁵
°C.	%	%	%	%
Control	0.000013	0.78	0.69	1.6
70	0.000022	—	—	1.6
75	0.000031	—	—	1.6
80	0.000032	0.79	0.67	1.6

¹ The heat-treated samples were raised to the indicated temperatures in 24 to 28 minutes, held at the specified temperature for 30 minutes, and cooled to 25°C. in 30 minutes.

² Single determination.

³ Mean of six determinations, three on one weighed sample and three on another.

⁴ Mean of three determinations or the hydrolyzate from one weighed sample.

⁵ Mean of duplicate determinations on separately weighed samples.

The titratable sulfhydryl groups decreased progressively as the heat-treatment was made more drastic. However, no change occurred in the cysteine content as determined in the hydrolyzate. In fact, all of the nonmethionine sulfur of the protein appeared to be in the form of cysteine regardless of heat-treatment. This result was very surprising in view of the reports of Kassell and Brand (21) and Brand *et al.* (7) that the major portion of the nonmethionine sulfur is present as cystine in "lactalbumin" and β -lactoglobulin respectively. The possibility that this apparent anomaly was due to reduction occurring during the hydrolysis or during the subsequent determinations was precluded by a test in which 22.5 mg. of cystine was added to the protein sample before hydrolysis and 94.5% of this amount was recovered by the analysis.

The data obtained for cysteine agreed satisfactorily with those obtained by others for the sum of the cysteine and cystine in milk serum proteins (5, 6). The methionine percentage is in reasonable agreement with other data (5, 6) and the sum of the cysteine and methionine sulfur represents about 91% of the total sulfur.

The amount of sulfur volatilized is of the same order of magnitude as that found by Townley and Gould (31) upon heating milk. For example, they found that an amount of sulfur equivalent⁴ to 0.000015% of the serum protein was volatilized during heating milk at 80°C. for 30 minutes. The results in Table II show that 0.000032% sulfur was volatilized by subjecting serum protein to the same treatment. The fact that a lower value is obtained by heating milk is in accord with the finding of Townley and Gould (33) that the presence of sugar reduces the volatilization of sulfides during heating.

The iodosobenzoate titration yields higher values for the sulfhydryl

⁴ Calculated on the assumption that one liter of milk contains 7.3 g. of serum protein.

content of unheated milk serum protein than has been obtained by the use of either thiamine disulfide or of ferricyanide as oxidants. Thus, Harland *et al.* (16) found that thiamine disulfide oxidized only a negligible number of groups in unheated milk serum protein. Crowe *et al.* (9) and Harland *et al.* (16) have reported values obtained on milk serum protein by the ferricyanide method which are equivalent to 0.05% and 0.29%⁴ cysteine respectively. A direct comparison of the three methods on a single sample of milk serum proteins is afforded by the data of Table III. These data were secured on a sample of protein prepared in the manner previously indicated. Portions were heat-treated in stainless steel tubes at 75°C. and 85°C. for 30 minutes in an atmosphere either of air or of nitrogen⁵ and the sulfhydryl determina-

TABLE III
EFFECT OF HEAT-TREATMENT ON THE SULFHYDRYL GROUPS IN MILK SERUM PROTEIN AS DETERMINED BY VARIOUS METHODS¹

Heat-treatment for 30 min. °C. Control	--SH groups (as cysteine) by various methods		
	Thiamine disulfide %	Ferricyanide %	o iodosobenzoate %
	0.02	0.09	0.63
HEATED IN AIR			
75	0.08	0.18	0.60
85	0.12	0.21	0.32
HEATED IN NITROGEN			
75	0.10	0.25	0.67
85	0.20	0.34	0.68

¹ Sol of protein was buffered at pH 6.6 with phosphate buffer having an ionic strength of 0.1. The protein concentration of the sol was 5.9 g. per liter.

tions were made immediately after heating and cooling. Comparison of results obtained by the three methods on the unheated samples suggests that the sulfhydryl groups of the protein are of graded reactivity or availability. Thus the number of groups oxidized depends on the oxidation-reduction potential of the oxidant and on the conditions employed.

Heat-treatment of the serum protein caused a decrease in the number of groups titratable by iodosobenzoate. On the other hand,

⁴ These values were computed from the data graphed in Fig. 6 of the paper by Crowe *et al.* (9) and in Fig. 3 of that of Harland *et al.* (16). The sample used by the latter was prepared from nonfat dry milk solids spray-dried from milk that had been preheated at 63°C. for 30 minutes; this treatment undoubtedly explains its higher capacity to reduce ferricyanide. Two other samples of serum protein isolated from fresh skim milk and analyzed by this method in the laboratories of the Division of Dairy Husbandry yielded values equivalent to 0.09 and 0.10% cysteine respectively.

⁵ This sample was prepared and heat-treated by H. A. Harland. The thiamine disulfide and ferricyanide determinations were also performed by him.

it has been shown that heat-treatment results in increases in the groups available to thiamine disulfide and ferricyanide (9, 16). The appearance of groups which reduce thiamine disulfide coincides with the appearance of a positive nitroprusside test. Evidently a small number of groups are made more active than were any groups originally.⁷

Obviously, the decrease which heat-treatment produces in the power to reduce iodosobenzoate cannot be due to loss of the insignificant amount of sulfur volatilized during heating. Another possibility is that the decrease in titratable sulfhydryl groups represents oxidation during or following heating. This possibility gains support from the facts that the groups which reduce thiamine disulfide are known to be susceptible to oxidation, and that in the present study, the protein sols for which data are reported in Tables I and II were protected from oxygen during the heat-treatment but were exposed to air in the subsequent dialysis and storage prior to analysis. It gains even more support from the data of Table III which show definitely that when the titration is performed immediately after heating and cooling the decrease in groups available to o-iodosobenzoate does not occur in the absence of oxygen during heat-treatment. However, the failure to detect cystine in hydrolyzates of either unheated or heated samples seems to preclude the possibility of explaining the decrease in titer on the basis of oxidation of sulfhydryl to disulfide linkages. This whole question of the possible role of oxidation is receiving further study at present.

In addition to oxidation, heat-treatment undoubtedly causes an unfolding of the protein molecules such that, upon cooling, the molecule refolds or agglomerates with other molecules in such a way that groups originally available to oxidation by iodosobenzoate are made sterically unavailable. This affords an alternate or additional explanation for the decrease in titration.

It is not known, of course, to what extent the changes produced by heat are due respectively to interactions among different species of protein micelles, to interactions among micelles of a given species, and to strictly intramolecular rearrangements. Since milk serum protein is a mixture of several electrophoretically different components (10, 29), the possibility of interaction is evident. Unpublished observations by one of us (R. J.) indicate that heat-treatment of milk serum protein in phosphate buffer at pH 6.9 and an ionic strength of 0.1 at 100°C. for 30 minutes causes the mixture to become electrophoretically more homogeneous, thus supporting the hypothesis that interaction can and does occur. On the other hand, Briggs and Hull (8) reported

⁷ The maximum observed by Harland *et al.* (16) was attained on heating at 95°C. for 2 minutes. It was equivalent to 0.33% cysteine.

that continuous aggregation occurs during heat-treatment of purified β -lactoglobulin. A few titrations made by the iodosobenzoate method on some of the β -lactoglobulin samples prepared by Briggs and Hull indicated that a decrease in titratable sulfhydryl groups is produced by heat-treatment of this rather homogeneous protein.

There is no assurance that the changes that have been found to occur in sols of milk serum protein in phosphate buffer are duplicated when these proteins are heated in their natural environment in milk. If oxidation is indeed a significant factor in the heat-induced changes, then the alterations produced in milk heated in the presence of air may be different from those recorded in this paper where air was excluded, at least during the heating process. In milk, moreover, the protein sulfhydryl groups may react with other nonprotein constituents. It has been demonstrated that when lactose is present in the system, heating produces an interaction between the proteins and lactose which results in a larger increase in capacity to reduce ferricyanide than that resulting from heating the protein alone (9, 16). Interestingly enough, lactose either retards the liberation by heat of groups that reduce thiamine disulfide or reacts with some of such liberated groups (16).

The decrease which heating produces in sulfhydryl groups titratable with iodosobenzoate coincides with the loss of dough-softening action which was shown in a previous paper to occur upon heating of sols of milk serum protein. Thus, these data agree with the suggestion of Stamberg and Bailey (30) that heat-treatment reduces the over-all activity of the sulfhydryl groups of milk serum protein and that this in turn causes the loss of dough-softening action and the improvement in baking quality of nonfat dry milk solids.

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BIOLOGICAL VALUE OF THE PROTEIN AND THE MINERAL, VITAMIN, AND AMINO ACID CONTENT OF SOYMILK AND CURD¹

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ABSTRACT

Soymilk was prepared by soaking the beans in water overnight followed by draining and grinding. Water was then added, the whole heated to 200°F., and solid matter filtered off. The curd was precipitated from the milk by the addition of magnesium chloride. Soymilk powder was prepared from the milk in a spray drier. The composition was then determined. The biological value of the protein of these products was assayed by the rat growth and nitrogen retention methods. The soymilk was 80% as efficient as whole milk powder, whereas the comparative value for the whole bean and curd was 75%. Supplementing soybean protein with sesame seed raised the value to 94%. The soymilk and curd contained approximately 0.25 to 0.34% of calcium, 0.01 to 0.015% of iron, and 0.8 to 1.0% of phosphorus. The curd had a higher percentage of these minerals than the original bean. Soymilk contained 11.8 μ g. thiamine, 4.6 μ g. riboflavin, and 29.0 μ g. nicotinic acid per g.; the curd 3.9 μ g. thiamine, 3.7 μ g. riboflavin, and 5.5 μ g. nicotinic acid per g. These figures were calculated on a moisture-free basis. The milk was a better source of these vitamins than the original bean. Soymilk contained 1.4% methionine and 2.4% of cystine; the corresponding values for the curd were 1.2 and 1.2% respectively.

Soybeans have long been regarded as a nutritious food. They are not only unusually high in protein (40%) and oil (20%), but are also good sources of the minerals—iron, calcium, and phosphorus—and the B vitamins (Payne and Stuart, 29).

Numerous investigations have been conducted on the biological value of soybean proteins. Work on men and dogs (25), rats (5, 18, 27, 28), and chicks (1, 2, 14) has indicated that proteins of adequately heated soybeans are high in value.

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Sprague (34) reported that soybeans contain substantial quantities of thiamine (0.8 to 1.3 mg. per 100 g.), niacin (4.8 to 9.0 mg. per 100 g.), and riboflavin (0.3 to 0.4 mg. per 100 g.). The results obtained by Burkholder (7) were in good agreement with these findings.

Bailey, Capen, and LeClerc (4) gave the following percentages for the mineral content of soybeans on an air-dried basis: potassium 1.9, sodium 0.3, calcium 0.2, magnesium 0.2, phosphorus 0.6, sulfur 0.4, and chlorine 0.02. Sherman, Elvehjem, and Hart (32) found that of the 0.01% of iron present in roasted soybeans 80% was available to the animal organism.

In this country soybeans are used mostly for animal feeds, and only small amounts are consumed as human food. Because of the high nutritive value, attempts are being made to adapt them to the American diet.

In the Orient soybeans have served as the sustaining food for centuries and have long been favored as "poor man's meat." In these countries they are consumed chiefly in the form of soymilk and soybean curd. Soymilk is a water extraction from soybeans, similar in composition and appearance to cow's milk, and the curd, produced by precipitating the protein from soymilk, has a texture somewhat like cream cheese.

Despite the fact that considerable work has been done on the nutritive value of soybeans, relatively little has been reported on soymilk and curd. The purpose of this paper was twofold: (1) to compare the biological value of the protein of the soymilk and curd with that of the original bean and whole milk powder and (2) to determine the vitamin, mineral, and amino acid content of these products.

Part I. Biological Value of Soybean Products

Cahill *et al.* (8) worked on human adults and found that soymilk was utilized for maintenance as well as cooked soybeans. Cheng (9) using the nitrogen-balance method with puppies observed that the biological value of soybean curd was only 70% as efficient as that of the raw bean. His results indicated that the value of the protein in the raw and cooked bean was about the same; a finding contrary to that of many workers. Desikachar (12) compared the biological value of soymilk protein with cow's milk and found that the former was about 90% as efficient as the latter.

In the present work rats were used to compare the protein efficiency of soymilk and curd with cooked bean and whole milk powder by the growth and nitrogen retention method.

Materials and Methods

Preparation of Soymilk and Soybean Curd. Mature, air-dry, Funk Delicious soybeans were used for all experiments. The milk was prepared according to the traditional methods used in China. The beans were soaked with four volumes of water overnight at room temperature (22°C.); they absorbed about one and one-half volumes of water. They were drained and ground. A quantity of water equivalent to four and one-half times the weight of the original dry bean was added and the mixture heated to 95°C. Milk was then obtained by filtering through a double thickness of cheese cloth.

The method of preparation used in France as described by Chin (10) was also tried, but the results were not as satisfactory. Since the bean was ground without previous soaking in this case, it was brittle and broke into particles of various sizes, some of which passed through the cheese cloth during filtration and a suspension instead of an emulsion resulted. Grinding the soaked bean produced flakes rather than powdery particles. Proximate analyses were carried out on the soybeans and the products (Table I).

TABLE I
COMPOSITION OF SOYBEANS, MILK, AND CURD

Constituents	Soymilk		Soybean curd		Soybean	
	Wet basis	Moisture-free basis	Wet basis	Moisture-free basis	Wet basis	Moisture-free basis
Moisture	% 91.0	% —	% 76.5	% —	% 5.3	% —
Protein ¹	4.4	48.4	15.0	63.7	41.4	43.7
Fat (ether extract)	1.7	19.0	7.1	30.0	21.0	22.2
Ash	0.5	5.7	0.9	3.6	4.6	4.9
Carbohydrate (by difference)	2.4	26.9	0.6	2.7	27.6	29.2

¹ Calculated using a factor of 6.25.

Animal Assay. Soymilk powder, soy curd, and soybeans were studied. The soymilk powder was prepared by drying the milk in an air spray dryer to powder form. The curd was frozen and dehydrated at 60°C. Since maximal biological value of soybean protein is obtained by autoclaving at 110°C. for 30 minutes (Evans and McGinnis, 14), beans were treated at this temperature for this period of time. The autoclaved curd was prepared in a similar manner. All dried products were ground, then analyzed for moisture and nitrogen using the Kjeldahl-Gunning-Arnold method (3). The quantity of each required to furnish a protein level ($N \times 6.25$) of 10% was used in

making the diets (Table II). Due to a high methionine content, sesame seed was added as a supplement to the autoclaved bean and curd diets. A diet containing whole milk powder at a 10% protein level was the standard for comparison.

TABLE II
COMPOSITION OF DIETS

Ingredients	Whole milk powder	Soy milk powder	Auto-claved soybean	Soybean curd	Auto-claved curd	Autoclaved soybean plus sesame meal	Curd plus sesame meal
	%	%	%	%	%	%	%
Whole milk powder	37.5	—	—	—	—	—	—
Soy milk powder	—	23.2	—	—	—	—	—
Autoclaved soybean	—	—	26.6	—	—	17.7	—
Dried soybean curd	—	—	—	17.9	—	—	12.0
Autoclaved curd	—	—	—	—	17.9	—	—
Sesame meal	—	—	—	—	—	7.1	7.1
Starch	60.0	68.6	66.5	74.8	74.9	68.9	74.4
Hydrogenated fat	—	5.7	4.4	4.8	4.7	3.8	4.0
Hubbell salts ¹	2.5	2.5	2.5	2.5	2.5	2.5	2.5

¹ Hubbell *et al.* (20).

Three times a week each rat received as a supplement 1 ml. of a vitamin solution containing thiamine hydrochloride 50 μ g., nicotinic acid 580 μ g., inositol 130 μ g., choline hydrochloride 20 mg., riboflavin 50 μ g., pyridoxine 50 μ g., calcium pantothenate 50 μ g., p-aminobenzoic acid 250 μ g., and $\frac{1}{3}$ ml. of a fat-soluble vitamin solution containing vitamin A 1,000 I.U. and D 100 I.U., and mixed tocopherols 2 mg. per ml.

Young albino rats, 28 to 31 days of age, were selected and paired as to sex and size into seven groups. They were housed in individual cages, weighed three times weekly, and fed *ad libitum*. Trials were run for 5 weeks.

At the conclusion of the experiment animals were killed, the gastrointestinal tract removed, and the carcass digested in 9% hydrochloric acid over a steam bath for 48 hours. The digest was homogenized in a Waring Blendor, made to volume and nitrogen determined on aliquots. Nitrogen storage was obtained by subtracting the nitrogen content of a comparable group analyzed at the beginning of the experiment from that of the experimental group.

Results

Growth studies are summarized in Table III and protein retention in Table IV; a statistical comparison of the figures is shown in Table V.

Animals on the soy milk diet ate more than those receiving whole milk powder and as a consequence a significantly higher gain resulted.

TABLE III
GROWTH RESPONSE ON DIETS USED TO DETERMINE
BIOLOGICAL VALUE OF SOYBEAN PRODUCTS

Diets	Number of animals	Average food eaten daily per rat	Average total gain	Average gain per gram of protein	Average food required for gram of gain	Per cent ¹ efficiency
		<i>g.</i>	<i>g.</i>	<i>g.</i>	<i>g.</i>	
Whole milk powder	8	10.5	77.1 ± 3.6 ²	2.30 ± 0.06 ²	4.80 ± 0.12 ²	100
Soymilk powder	8	12.4	95.1 ± 6.9	1.88 ± 0.07	4.64 ± 0.17	82
Autoclaved soybean	8	11.8	80.9 ± 3.2	1.73 ± 0.05	5.13 ± 0.13	75
Soybean curd	8	10.7	71.0 ± 4.4	1.73 ± 0.05	5.31 ± 0.16	75
Autoclaved soybean curd	8	9.9	61.8 ± 5.1	1.59 ± 0.09	5.77 ± 6.34	69
Autoclaved soybean + sesame seed	8	12.0	99.8 ± 7.7	2.17 ± 0.09	4.34 ± 0.23	94
Soybean curd + sesame seed	8	11.3	91.0 ± 5.8	2.10 ± 0.10	4.45 ± 0.25	91

¹ Calculated from protein efficiency of each diet using whole milk powder as 100.

² Standard error.

However, the protein efficiency of the whole milk powder was higher than that of soymilk, bean, or curd; statistical analysis gave a "t" value of 4.6 for milk versus soymilk, 7.7 for milk versus soybean, and 7.5 for milk versus curd; these differences are highly significant. The soymilk appeared to be slightly better in quality than the whole bean. Growth on the soymilk diet was significantly better than on the curd, but this was not true of the efficiency. The protein efficiency of the whole bean and curd was the same. Autoclaving did not improve the curd. Jones and Widness (21) report a protein efficiency of 2.64 for skim milk powder and Block and Mitchell (6) cite a figure of 2.8, values higher than the one secured in this study with whole milk powder.

TABLE IV
NITROGEN RETENTION ON DIETS USED TO DETERMINE
BIOLOGICAL VALUE OF SOYBEAN PRODUCTS

Diets	Number of animals	Average nitrogen retention	Average retention per gram of nitrogen	Per cent ¹ efficiency
		<i>g.</i>	<i>g.</i>	
Whole milk powder	8	1.97 ± 0.13 ²	0.37 ± 0.01 ²	100
Soymilk powder	8	2.37 ± 0.19	0.29 ± 0.02	79
Autoclaved soybean	8	1.99 ± 0.01	0.27 ± 0.01	73
Soybean curd	8	1.80 ± 0.10	0.27 ± 0.01	73
Autoclaved soybean curd	8	1.48 ± 0.11	0.24 ± 0.01	65
Autoclaved soybean + sesame seed	8	2.60 ± 0.18	0.36 ± 0.02	97
Soybean curd + sesame seed	8	2.44 ± 0.12	0.35 ± 0.01	95

¹ Calculated using whole milk powder as 100.

² Standard error.

TABLE V
STATISTICAL COMPARISONS OF GROWTH AND NITROGEN
RETENTION ON VARIOUS DIETS

Diets compared	Weight gain (g.)		Gram gain per gram protein		Total nitrogen retention (g.)		Nitrogen retention per gram nitrogen	
	Mean difference	t ¹	Mean difference	t ¹	Mean difference	t ¹	Mean difference	t ¹
Milk vs. soymilk	18.0	2.32*	0.42	4.61**	0.40	1.84	0.08	3.95**
Milk vs. autoclaved soybean	3.8	0.79	0.57	7.70**	0.02	0.86	0.10	5.65**
Milk vs. curd	6.1	1.07	0.57	7.50**	0.18	0.96	0.10	6.76**
Soymilk vs. autoclaved soybean	14.2	1.88	0.15	1.76	0.39	1.80	0.02	1.05
Soymilk vs. curd	24.1	2.96**	0.15	1.70	0.58	2.71*	0.02	1.25
Autoclaved soybean vs. curd	9.9	1.83	0.00	0.00	0.19	1.07	0.00	0.00
Curd vs. autoclaved curd	9.2	1.37	0.14	1.31	0.31	1.69	0.03	2.66*
Autoclaved soybean + sesame seed vs. autoclaved bean	18.9	2.27*	0.44	4.26**	0.62	2.89*	0.09	4.68**
Curd + sesame vs. curd	20.0	2.74*	0.37	3.59**	0.64	3.43**	0.08	5.12**
Milk vs. autoclaved soybean + sesame	22.7	2.67*	0.13	1.20	0.63	2.94*	0.01	0.49
Milk vs. curd + sesame	13.9	2.04	0.20	1.64	0.47	3.83**	0.01	1.19

¹ Calculated from Fisher's formula (Snedecor, 33).

The addition of sesame seed to both autoclaved soybean and curd increased growth but not protein efficiency above that obtained on milk powder.

In general, the correlation between values resulting from growth, protein efficiency, and nitrogen retention was good with the exception that soymilk produced significantly greater growth than milk powder, but both the efficiency and nitrogen retention were significantly lower.

Part II. Vitamin Mineral and Amino Acid Content of Soybean Products

With the exception of the work of Miller (26) on the thiamine content of soybean curd, few references are found in the literature on this subject. According to his report only 20% of the thiamine in the bean was retained in the curd. Since soybeans are considered rich sources of B vitamins—thiamine, riboflavin, and nicotinic acid—retention in the milk and curd was investigated.

No information on the mineral content of soybean products was found. Because the content of phosphorus, calcium, and iron in soybeans is high, investigation of the retention of these nutrients in soymilk and curd was considered desirable.

Soybean and the products were analyzed for methionine since various workers have demonstrated that the amount of this amino acid limits the biological value of soybean protein.

Materials and Methods

Thiamine was determined by the method of Conner and Straub (11) modified to include extraction of the samples with isobutyl alcohol to remove nonthiochrome fluorescing materials (Harris and Wang, 17). The method of Peterson, Brady, and Shaw (30) was used for riboflavin, modified by the use of 0.1 *N* sulfuric acid for extraction of the samples. Nicotinic acid was determined by microbiological assay employing a dehydrated medium prepared by a commercial laboratory.⁴ Sodium hydroxide was used for extraction of the sample as described by Teply, Strong, and Elvehjem (35).

Minerals were determined on the dry sample by wet ashing with perchloric acid according to the method of Gerritz (16). Calcium was determined by ceric sulfate titration using the method of Kirk and Schmidt (23) with the modification of Kirk and Moberg (22). Fiske and Subbarow's colorimetric method (15) was employed for the determination of phosphorus. Iron was analyzed by the colorimetric method of Koenig and Johnson (24).

Methionine was assayed by two methods: the differential oxidation method of Evans (13), and the microbiological method of Henderson and Snell (19). Using the Evans procedure, some methionine apparently is oxidized along with the cystine during nitric acid digestion, resulting in low recovery for the former (90%) and high for the latter (110%); accordingly this digestion period was cut from 24 to 16 hours.

For microbiological assay, samples were prepared by hydrolyzing with 2 *N* hydrochloric acid in the autoclave at 15 lb. pressure for 5 hours. Longer treatment did not release more of the amino acids but resulted in some destruction. Henderson and Snell's medium was employed with the following modifications: 100 times as much folic acid was added to the medium in order to increase the acid production to a maximum at high levels of methionine when *Leuconostoc mesenteroides* was used as the assay organism and DL-glutamic acid was used instead of the L-isomer because the latter was found to contain traces of methionine which interfered with the determination. When these changes were employed, smooth standard curves with low blanks and high acid production were obtained repeatedly with *Leuconostoc mesenteroides* P-60⁴ and *Lactobacillus arabinosus* 17-5⁴.

Results

The results of vitamin, mineral, and amino acid analyses in the soybean and its products are shown in Tables VI, VII, and VIII. The retention of minerals and vitamins in these samples was compared on a moisture- and fat-free basis.

⁴ Difco Laboratories, Inc., Detroit, Michigan.

TABLE VI
THIAMINE, RIBOFLAVIN, AND NICOTINIC ACID
CONTENT OF SOYBEAN, MILK, AND CURD¹

Products	Moisture-free basis µg./g.			Moisture- and fat-free basis µg./g.		
	Thiamine	Riboflavin	Nicotinic acid	Thiamine	Riboflavin	Nicotinic acid
Soybean (raw)	8.7	3.2	15.9	11.3	4.1	21.0
Soymilk	11.8	4.6	29.0	14.8	6.1	35.0
Soybean curd	3.9	3.7	5.5	6.6	5.0	6.0

¹ Average of duplicate determinations.

TABLE VII
CALCIUM, IRON, AND PHOSPHORUS CONTENT OF
SOYBEAN, MILK, AND CURD

Products	Moisture-free basis %			Moisture- and fat-free basis %		
	Calcium	Iron	Phosphorus	Calcium	Iron	Phosphorus
Soybean (raw)	0.205	0.0078	0.57	0.263	0.0100	0.73
Soymilk ¹	0.195	0.0072	0.65	0.241	0.0089	0.80
Soybean curd ¹ (MgCl ₂ precipitated)	0.241	0.0105	0.80	0.344	0.0150	1.07
Soybean curd (CaCl ₂ precipitated)	0.690	—	—	0.990	—	—

¹ Analyses on soymilk and curd were run on pooled samples from several preparations.

TABLE VIII
METHIONINE AND CYSTINE CONTENT OF PROTEIN OF
SOYBEAN, MILK, AND CURD

Products	Differential oxidation method ¹		Microbiological method ¹	
	Methionine ²	Cystine ²	Methionine ²	
			Organism employed	
			<i>Leuconostoc mesenteroides</i>	<i>Lactobacillus arabinosus</i>
	%	%	%	%
Soybean (raw)	1.14	1.83	1.14	0.78
Soymilk	1.36	2.32	1.16	0.85
Curd	1.22	1.19	1.05	0.77
Sesame seed	3.35	2.74	—	—

¹ Calculated to 16% nitrogen.

² Average value of duplicate determinations.

Higher concentrations of the three vitamins were found in the soymilk. This could be anticipated due to their solubility in water. Only about one-half of the thiamine and one-fourth of the nicotinic

acid were retained in soybean curd. Probably these were lost by solution when the curd was separated from the whey. The riboflavin content was equivalent to that of the original bean.

The mineral content in soymilk was comparable to that of the bean, whereas higher concentrations of calcium, iron, and phosphorus were found in the curd. Since the dairy industry is not well developed in China, the adequacy of calcium in the diet has been considered one problem in the nutrition of the Chinese. In order to introduce more calcium, an effort was made to enrich the curd with this mineral. The soymilk was precipitated with a solution of calcium sulfate or chloride instead of magnesium salt. The resulting products apparently were not different in texture and taste, while the calcium content was raised from 0.24 to 0.69 g. per 100 g. Schroeder, Cahill, and Smith (31) reported that calcium from soymilk is 80% as available for human utilization as the calcium in cow's milk. The use of soybean curd precipitated by a calcium salt should be helpful in increasing the amount of this mineral in the Chinese diet.

The methionine content of soybean products as determined by the differential oxidation method agreed fairly well with the microbiological assay using *Leuconostoc mesenteroides*, but was higher than results obtained by using *Lactobacillus arabinosus*. High concentrations of sodium chloride resulting from the neutralization of hydrochloric acid used for digestion were not responsible for this difference. When casein was assayed for methionine using *Lactobacillus arabinosus*, low results were again obtained. From these findings the conclusion may be drawn that the use of *Leuconostoc mesenteroides* is preferable for methionine assays.

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EFFECT OF BISULFITE, ACETALDEHYDE, AND SIMILAR REAGENTS ON THE PHYSICAL PROPERTIES OF DOUGH AND GLUTEN¹

I. HLYNKA

ABSTRACT

Small amounts of sodium bisulfite produce a soft, sticky, and inelastic gluten; larger amounts destroy the gluten-yielding property of dough. Acetaldehyde can prevent bisulfite from affecting the gluten when the two reagents are incorporated simultaneously into dough, but it can also counteract existing effects in bisulfite-treated gluten. By means of bisulfite-acetaldehyde treatment some inferior dried glutes, which could not be reconstituted with water alone, were reconstituted to glutes of reasonably good physical quality. A large number of common reducing agents were found ineffective in producing in dough an effect similar to that of bisulfite. Bisulfite-like reactions were produced with cysteine, glutathione, and sulfide. But various differences occurred in effects of the reagents on gluten, and in the modification of effects caused by acetaldehyde. The results are discussed from the point of view of dissociation and formation of cross-linkages in a three-dimensional network of gluten. Either the disulfide may provide the necessary cross-linkages or carbonyl compounds may act as cross-linking agents between protein molecules.

Although elasticity is one of the basic and most characteristic properties of dough and gluten, our knowledge of its structural basis is meager. We know that the requisites for elasticity in polymers are, first, a long chain, and second, cross-linkages joining the linear polymer chains at intervals to form a three-dimensional network. The gluten proteins in dough undoubtedly provide the linear polymer in the polypeptide structure. However, little experimental evidence exists as to the identity of the cross-linkages, although the disulfide linkages are assumed to be involved (5, 11).

The most pertinent work on the disulfide cross-linkages in proteins has come from the laboratories of the Wool Industries Research Association (3). It has been shown that overnight treatment of wool with bisulfite (usually 13%), at room temperature, breaks the disulfide linkage to form a free thiol group (R-SH) and perhaps an unstable sulfenic acid (R-SOH) which adds sulfite to form cysteinyl S-sulfonate (RSSO₂ONa). Lindley (7) treated gliadin with a 25% sodium sulfite

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solution at room temperature overnight and obtained a fractionation similar to that for wool.

An alternate possibility is suggested by the work of Fraenkel-Conrat and Olcott (4) which showed that aldehydes can act as cross-linking agents between amide, amino, and guanidyl groups, on the one hand, and ammonia, primary amines, and amino acids, on the other. Gliadin treated with formaldehyde was found to bind large amounts of ammonia, amines, and amino acids. This information becomes more significant when considered together with the well-known tendency of carbohydrates to react, through the carbonyl group, with proteins in the so-called browning reaction. In other words, reducing carbohydrates may conceivably act as cross-linking agents in dough.

If it is assumed that the structural basis of elasticity in dough relates to cross-linkages between protein chains, it may be inferred that reagents that change the elasticity do so either by establishing or dissociating cross-linkages, or by splitting protein chains, i.e., by affecting protease activity. On these grounds, the chemists' chief problem appears to be that of elaborating hypotheses at the molecular level which are consistent with observations made on the properties of the dough and on the changes in these properties caused by certain reagents. Among the more interesting reagents that affect dough properties are bisulfite and acetaldehyde. Small amounts of bisulfite produce a soft, sticky, and inelastic dough; acetaldehyde reverses the effect produced with bisulfite. The influence of these and other closely related reagents on dough and gluten is described in this paper and discussed in terms of hypotheses relating to the molecular reactions that may be involved.

Methods and Results

The Effect of Bisulfite and Acetaldehyde on Dough and Gluten. Small amounts of bisulfite markedly modify the elasticity of dough and gluten and the behavior of dough in the gluten-washing process. Changes in these properties were therefore used in assessing the effect of adding bisulfite and acetaldehyde to dough or gluten.

Gluten-washing experiments according to the A.O.A.C. method (1) were carried out on a casual sample of flour in which 10 mg. sodium bisulfite per 25 g. flour were incorporated into the dough. This amount of bisulfite was sufficient to destroy the gluten-yielding property of dough when washed in the usual way by hand; i.e., no elastomer was formed. In parallel experiments in which various amounts of acetaldehyde solution were added simultaneously with bisulfite, elastic gluten was readily obtained. The minimum amount of acetaldehyde required to prevent the action of 10 mg. bisulfite in dough was approximately 10 mg. On a mole-equivalent basis the ratio is 2.4 to 1.

Variations of the above experiments showed that soft, sticky, and inelastic gluten could be obtained by washing bisulfite-treated dough in a beaker with a stirring rod under a stream of cold water. This gluten completely recovered its normal handling characteristics and elasticity upon resting for an hour or two in water containing acetaldehyde up to 0.5%. Again, good gluten obtained in a normal way deteriorated rapidly in quality when placed in a solution of bisulfite, but recovered when subsequently placed in an acetaldehyde solution. Alone, acetaldehyde had a slightly toughening effect on gluten. The various reactions could be speeded up either by working the gluten with a stirring rod or by pinching off small pieces into a solution containing the reagent. Moreover, the effects of successive treatments with bisulfite and acetaldehyde have been demonstrated several times on the same sample of gluten.

Thus, acetaldehyde not only prevents bisulfite from affecting gluten when the two reagents are incorporated simultaneously into dough, but it also counteracts effects already caused by bisulfite. This observation was sufficiently new and interesting in itself to warrant further exploration. In addition, through the mechanism of the bisulfite and acetaldehyde reactions, information was sought on the identity of the cross-linkages which are responsible for the elastic property of dough and gluten.

Bisulfite and Acetaldehyde Experiments with Deteriorated Gluten. Treatment with bisulfite and acetaldehyde produced unusual results with samples of dried gluten which had deteriorated with age. Several preparations of varying quality and history were treated as follows: Five-gram amounts were reconstituted with water and allowed to rest for one hour. They were then treated for one hour with bisulfite alone, or with acetaldehyde alone. In addition, some samples were treated with bisulfite and one hour later with acetaldehyde and examined at the end of another hour. All samples were worked several times during the course of the treatments. The amounts of reagents were 1 ml. of a solution containing 5 mg. bisulfite per ml. and 2 ml. of 5% acetaldehyde solution. Results shown below were selected for greatest response to the bisulfite-acetaldehyde treatment; data on samples showing no response are omitted. Zero rating was assigned to gluten which formed only a "mush" and +++ to good, elastic gluten.

Gluten No.	Treatment			
	Water	Bisulfite	Acetaldehyde	Bisulfite-acetaldehyde
1	0	0	+	+++
2	0	+	+	++++
3	0	+	+	+++

It is remarkable that glutens which rated zero when reconstituted with water alone were brought to a +++ rating with the bisulfite-acetaldehyde treatment. This improvement, however, was not permanent and the glutens reverted to their impaired condition on continued washing under the tap.

The Effect of Reagents Possessing Action Similar to Bisulfite and Acetaldehyde. Substances containing the sulfhydryl group have been shown to affect dough and gluten in a way similar to bisulfite (2, 7, 10, 11). Preliminary comparative experiments were carried out with cysteine, glutathione, and sodium sulfide, and the combined effects of acetaldehyde and each of these reagents were also determined.

When 25 mg. of cysteine hydrochloride were incorporated into dough (25 g. flour) no gluten could be recovered by the usual washing procedure. With smaller concentrations, sticky, soft, and inelastic gluten was obtained. This gluten, however, showed some improvement in physical properties with continued washing (an effect which was not apparent with bisulfite-treated gluten). The action of cysteine was inhibited by simultaneous addition of acetaldehyde.

When normal gluten from 25 g. flour was placed in 75 ml. water containing 25 mg. cysteine hydrochloride, its surface soon became gelatinous. After several hours, the gluten became soft, inelastic, and sticky. This impaired gluten recovered its physical properties when subsequently placed in water containing 1 ml. of 10% acetaldehyde solution. A slight improvement was also obtained in water alone.

Similar experiments were made with glutathione. Increments up to 40 mg. were incorporated with 25 g. flour into dough. Reduced yields of impaired and sticky gluten were obtained. This, like cysteine-treated gluten, showed a slight improvement on prolonged washing. However, addition of acetaldehyde simultaneously with glutathione gave no recovery of gluten by washing; in this respect glutathione is dissimilar to cysteine.

Gluten placed into 75 ml. water containing 40 mg. glutathione became slippery and glutinous on the surface and after several hours became soft, inelastic, and sticky. This impaired gluten showed slight recovery in water but disintegrated rapidly in dilute solutions of acetaldehyde. Here, again, glutathione is dissimilar to cysteine.

Disintegration was also noted in comparative experiments with papain in which greater destruction was obtained in doughs containing acetaldehyde and papain than in those containing papain alone.

Sodium sulfide was required in relatively large amounts to produce effects similar to those of cysteine and glutathione. Dough made from 25 g. of flour with 150 mg. of sodium sulfide still gave recovery of a weakened gluten, which seemed to improve with washing. When small

pieces of normal gluten were pinched off into a 1% buffered solution of sodium sulfide, the gluten became very slippery, a great deal seemed to disperse, and only a small amount of inelastic, sticky gluten could be gathered together. This impaired gluten recovered almost entirely in acetaldehyde solutions, but only slightly, if at all, in water.

The effect on bisulfite-treated dough and gluten of several substances which were expected to have the same action as acetaldehyde was also ascertained. Formaldehyde, acetone, glucose, and wheat starch were found to possess some activity in preventing or reversing the effect of bisulfite on gluten. Acetaldehyde was approximately twice as active as formaldehyde and about 100 times as active as acetone when these reagents were incorporated into dough simultaneously with bisulfite. A small positive effect was also noted with glucose and wheat starch when they were first worked into bisulfite-impaired gluten and then washed out again.

The Action of Reducing Agents. Because the effect of bisulfite, cysteine, glutathione, and sulfide has been attributed to their reducing action (8, 10, 11), a number of other reducing agents were studied. The destruction of gluten-yielding ability of dough in the gluten-washing test already described was used as the basis of comparison. The reducing agents listed were tried in equimolar, 5 times, and 25 times the molar concentration of bisulfite required to destroy gluten in the gluten-washing test.

The following chemicals did not produce a similar effect to that of bisulfite as judged by the gluten-washing test. The gluten could be washed out normally; but at higher concentrations of some reagents, a short, harsh texture, rather than a soft, pliable, and smooth one, was obtained.

ascorbic acid
calcium metal
(50 mg./25 g. flour)
cuprous chloride
ferrous ammonium sulfate
ferrous chloride
ferrous sulfate

hydroquinone
mercurous chloride
neutral red
potassium ferrocyanide
pyrogalllic acid
sodium thiosulfate
stannous chloride

Those chemicals which had a strong acid reaction, such as ascorbic acid and stannous chloride, were used in 15 ml. of phosphate buffer solution with which the dough was made up. Others were used in aqueous solutions.

A few values for standard oxidation-reduction potentials show that strongly reducing substances are included in the above list. For example, mercurous-mercuric system has an E_0 value of -0.92 volts; ferrous-ferric, -0.74 volts; ferrocyanide-ferricyanide, -0.49 volts; and stannous-stannic, -0.13 volts. Since a variety of reducing agents

have no effect on gluten, the effect of bisulfite appears to involve some more specific reaction than mere reduction.

Farinograms and Extensograms on Doughs Treated with Bisulfite and Acetaldehyde. The criteria so far used in assessing the effects of vari-

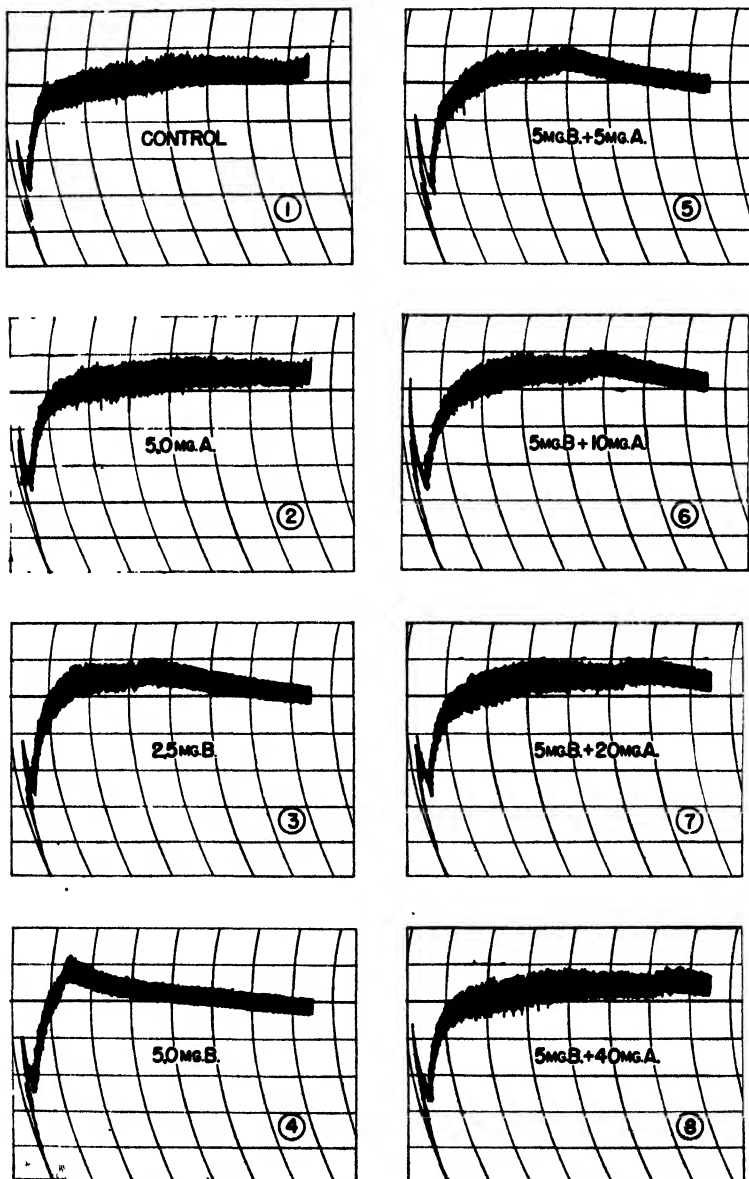


Fig. 1. Farinograms showing the effect of bisulfite and acetaldehyde on dough: B = bisulfite, A = acetaldehyde in mg.%.

ous reagents on dough and gluten involve subjective judgments which are not very precise. Accordingly, several experiments were carried out on the farinograph and extensograph which are capable of yielding data that are more precise and objective, though still empirical. Unfortunately, since it was not experimentally feasible to add acetaldehyde to the doughs after they were formed, no tests were conducted to show that the effect of bisulfite could be reversed with acetaldehyde. Experiments were therefore confined to tests in which the action of bisulfite in dough was merely prevented by the simultaneous addition

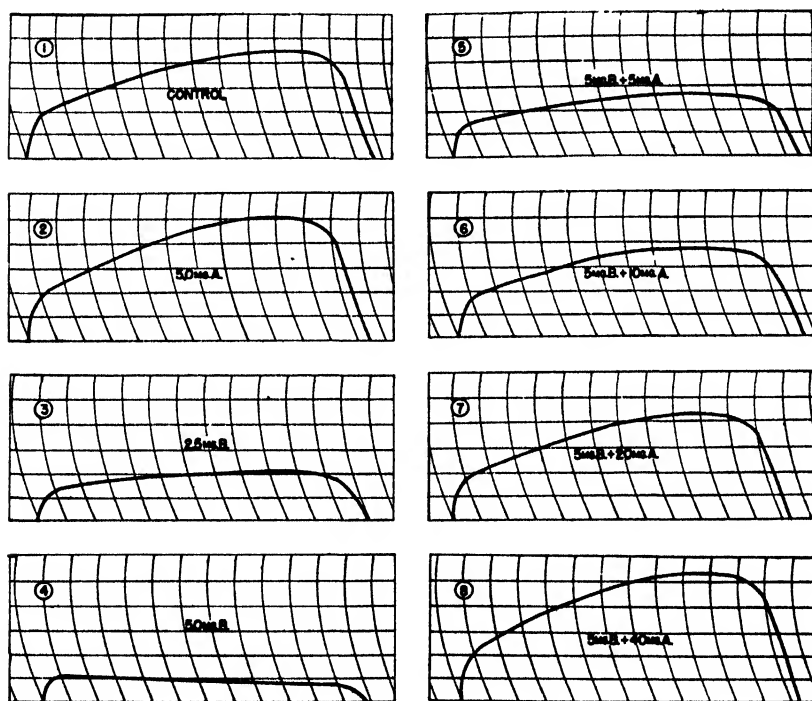


Fig. 2. Extensograms showing the effect of bisulfite and acetaldehyde on dough: B = bisulfite, A = acetaldehyde in mg. %.

of acetaldehyde. It should also be borne in mind that the farinograph and the extensograph measure physical properties of dough. Therefore, the results obtained previously in tests which depend on the gluten-yielding ability of dough when washed, and on the physical properties of gluten obtained, are not necessarily comparable with farinogram and extensogram results.

The effect of bisulfite and acetaldehyde on the physical properties of dough is shown by farinograms in Fig. 1. The strength of the flour represented by farinogram 1 is reduced successively by the effects of

2.5 and 5.0 mg. % bisulfite shown by farinograms 3 and 4. Farinogram 4 is typical of a flowy but inelastic dough such as is obtained from a soft wheat flour. Now, although acetaldehyde alone has no appreciable effect on the dough, as shown by farinogram 2, it can nullify the effect of bisulfite when the two reagents are added simultaneously; farinogram 5 in which the dough was treated with 5 mg. % bisulfite plus 5 mg. % acetaldehyde is approximately the same as farinogram 3 in which the dough contained only 2.5 mg. % bisulfite. From this comparison it may be estimated that approximately 4 moles of acetaldehyde is equivalent to 1 mole of bisulfite. This equivalence ratio is higher but of the same order of magnitude as that obtained by the gluten-washing technique. Farinograms 7 and 8 show practically complete inhibition of the bisulfite effect by acetaldehyde; the dough retained normal elasticity and handling characteristics.

Extensograms were also made with the same concentration of reagents as for farinograms. These are shown in Fig. 2. Resistance to extension, represented by the height of the extensogram, is reduced from 450 units to 200 and 100 units for the 2.5 and 5.0 mg. % bisulfite levels. Extensogram 4 is typical of extensible but inelastic dough, characteristic of soft wheat flour. Curve 7 shows that the action of 5 mg. % bisulfite has been prevented with 20 mg. % acetaldehyde. Curves 2 and 8 show that acetaldehyde has a small positive effect in increasing the resistance of dough to extension. It is interesting to point out also that practically no change in extensibility of the dough took place in the bisulfite and acetaldehyde treatments.

Discussion

The foregoing data establish that the damage from bisulfite action to gluten can be reversed with acetaldehyde, although these compounds do not form an oxidation-reduction system. Subsidiary to this finding is that only a restricted group of reducing agents have a bisulfite-like effect on dough, and that there are differences in action among bisulfite, cysteine, glutathione, and sodium sulfide. These topics will be briefly discussed, and their relation to hypotheses about cross-linkages as the structural basis of elasticity in dough and gluten will be indicated.

Firstly, the reversibility of bisulfite damage in gluten does not appear to be consistent with the hypothesis that proteolysis is involved, as has been suggested by Jørgensen (6) and by Balls and Hale (2). Secondly, the effect of bisulfite on gluten cannot be adequately explained in general terms of oxidation-reduction, since the bisulfite and acetaldehyde used in our studies do not constitute an oxidation-reduction system. This also follows from the finding that many re-

ducing agents do not have a bisulfite-like effect on dough. It appears that the effect is restricted to bisulfite, sulfhydryl compounds, and perhaps cyanide.

Certain differences in the effects on gluten produced by bisulfite, cysteine, glutathione, and sulfide suggest that more than one reaction is involved. Bisulfite produces a characteristically weak and very sticky gluten; sulfide produces gluten with a slippery gelatinous surface; and the remaining compounds produce effects intermediate between these two. The effect of acetaldehyde on glutes treated with different reagents also shows great differences. Acetaldehyde counteracts or reverses the effects of bisulfite, cysteine, and sulfide, but not those of glutathione or papain, with which a different reaction comes into prominence. At very small concentrations, acetaldehyde may partly reverse glutathione damage in gluten, but this reaction is obscured by a disintegrating action which becomes pronounced at concentrations of approximately 0.5%. Acetaldehyde has also a strong disintegrating effect on papain-treated gluten. Proteolytic mechanism for the effect of glutathione on gluten is therefore not excluded. Finally, there is a difference in reactivity of the different reagents. On a mole-equivalent basis, bisulfite is the most effective, followed by cysteine and glutathione, with sodium sulfide very much less effective.

We now come to an interpretation of these summarized findings in terms of the hypotheses set out in the beginning. So far it has been indicated that the loss of characteristic elasticity when gluten is treated with bisulfite cannot be explained on the basis of proteolysis. It has also been suggested that more than one reaction is involved in the action of bisulfite, cysteine, glutathione, and sulfide on gluten. Let us now consider to what extent these reactions can be identified.

Elsworth and Phillips (3) definitely showed that bisulfite dissociates disulfide linkages in wool. Patterson *et al.* (9) pointed out that wool is also susceptible to sulfhydryl compounds, sulfide, and cyanide, the same group to which gluten is susceptible. It may be inferred from studies on wool and from similar experiments on gliadin (7) that the action of bisulfite on gluten is on the disulfide linkages, and from the reported data on reducing agents that this reaction is disulfide fission rather than an oxidation-reduction reaction. Acetaldehyde thus appears to prevent or reverse the reaction by binding the bisulfite and removing it from the reacting system. Changes in elastic properties may thus be explained by fission and reconstitution of the disulfide cross-linkages. On this basis, a definite correlation would be expected between disulfide content of gluten and its elastic properties. No publication establishing that such a correlation exists has come to the author's attention.

The alternate or additional possibility of forming cross-linkages between carbonyl groups of reducing carbohydrates, on the one hand, and amine, amide, and guanidyl groups of proteins, on the other, must also be examined. It may not be altogether co-incidental that compounds possessing a bisulfite-like effect are also characterized by their ability of adding to carbonyl groups. They could thus free protein from combination in a protein carbohydrate-protein complex. Again, acetaldehyde would simply remove bisulfite from the reacting system by forming an addition compound with it. It is interesting in this connection that bisulfite, which is the most effective compound in preventing browning reaction between carbohydrates and proteins, is also most effective in its action on gluten properties. It is realized, of course, that the effectiveness of bisulfite in preventing browning reaction can be explained in other ways, but the above observation is none the less suggestive.

At this stage, there is insufficient evidence to exclude either theory of cross-linkages in gluten. It is likely that both play a part. Future work, however, may eventually determine the part that these or other reactions play in the changes that occur in the physical properties of dough.

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EFFECT OF COMMERCIAL FERTILIZERS AND GREEN MANURE ON YIELD AND NUTRITIVE VALUE OF WHEAT ¹

I. NUTRITIVE VALUE WITH RESPECT TO TOTAL PHOSPHORUS, PHYTIC PHOSPHORUS, NONPHYTIC PHOSPHORUS, AND CALCIUM CONTENT OF THE GRAIN

G. S. BAINS ²

ABSTRACT

Studies were made on the effect of potassium nitrate, superphosphate, and superphosphate plus ammonium sulfate in doses of 25 lb. phosphoric acid (P_2O_5) and 60 lb. nitrogen per acre with and without green manure on the yield and nutritive value of wheat. The phosphatic treatments increased the yield by 38 to 48% over the control. As the phytic phosphorus was found to be positively correlated with calcium content ($r = +0.74$), the nonphytic phosphorus content was taken as the criterion of nutritive value. The percentage proportion of nonphytic phosphorus to total phosphorus ranged from 23.4% in grain of plots fertilized with superphosphate plus ammonium sulfate and green manure to 37.0% in grain of the plots treated with superphosphate plus green manure. The availability of phosphorus as determined by *in vivo* experiments, however, exceeded the corresponding nonphytic phosphorus values by 27 to 32%.

The importance of calcium and phosphorus in nutrition is a well-recognized fact. The distribution of various minerals, inclusive of calcium and phosphorus, in plants, vegetables, and food grains and the effect of various factors, such as climate, irrigation, soil, and manures, on the composition of food products with particular reference to the total phosphorus and calcium content in grains has been investigated rather exhaustively. Greaves and Hirst (6) reported great variation in the calcium and phosphorus content of wheat grains due to differences in variety, soil type, and irrigation. Murphy (17) observed that manuring with superphosphate increased the total phosphorus content of wheat.

Observations of similar nature have also been reported in the case of forage crops. It is well known that phosphatic fertilizers cause a marked increase in the total phosphorus content of crops and plants

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grown on phosphate-deficient soils, but accurate information is lacking as to whether this increase is in organic or inorganic form and how such variations are associated with the calcium content of the grains. Numerous workers (3, 4, 10, 16, and 22) demonstrated the beneficial effect of green manuring in releasing and making available calcium and iron to the plants from the bound and unavailable forms. However, there is little information concerning the effect of green manure applied in conjunction with artificial fertilizers, especially those containing phosphorus, on the total phosphorus and calcium content of the grains; the effect of such a combination on calcium and on the various forms of phosphorus present in the grain has not been studied.

Considerable literature is extant to show that estimations of calcium and phosphorus content of the grain may be an unreliable index of the value of grain as a source of these elements.

There are a number of organic phosphorus compounds, such as phytin, phospholipids, hexose-phosphates, nucleic acid, and phosphoproteins, etc., which occur in plants and plant seeds. Of these, phytin is of considerable interest. Posternak (20) observed that phytin phosphorus constituted 70 to 90% of the total phosphorus of the wheat grain. His observations have been corroborated by the work of Andrews and Bailey (2), Knowles and Watkin (11), and Harris and Mosher (7), that some 40 to 70% of the total phosphorus in cereal grains is present in the form of phytin. Recent investigations throw considerable light on the probable role of phytin in human nutrition. A comprehensive account of the nature and significance of this compound has been given by Harrison and Mellanby (8), who stated that phytic acid renders calcium unavailable. In a rachitogenic cereal, phytic acid immobilizes all or almost all of the calcium contained in the cereal by converting it into insoluble calcium phytate, which is not absorbed, while the excess over and above that required to precipitate the calcium of the cereals can exert an additional anticalcifying action by precipitating further amounts of the calcium from the noncereal part of the diet. This view fits in with the observations that the anticalcifying effect of the cereals can be antagonized by feeding extra calcium. In some earlier investigations, Mellanby (13, 14) found that the addition of calcium salts, such as calcium carbonate and calcium phosphate, to a diet largely composed of oat meal reduces its rachitogenic effect. These results have since been confirmed by Mottram and Palmer (15, 18) by using calcium lactate.

Cereals constitute a major portion of the diet of millions of people in India. The consumers, for want of adequate milk supply, mainly depend upon them for their requirements of essential minerals such as calcium and phosphorus. The importance of cereals as the main source

of these elements assumes greater significance in view of the acute shortage of food grains in the country, especially when it is also known that a greater portion of the total phosphorus of the grain is present in the relatively unavailable phytic form. The extent to which commercial fertilizers in conjunction with green manure can increase yield and improve the nutritive value of wheat, which is one of the major food crops of the country, was, therefore, considered worth while to investigate. Besides recording yield data, determinations were made of the calcium, total phosphorus, and phytic and nonphytic phosphorus content of the grain. *In vivo* studies have also been carried out to find out the influence of variations in the phosphorus content of the grain, due to fertilization, on the availability of this mineral by the "balance sheet" method.

Material and Methods

The material employed in the present investigation consisted of wheat C 409 (1938-39) crop sown under field conditions and was obtained from the replicated plots of the four continuous randomized blocks of the manurial experiments underway at the Agricultural Experimental Station, Rawalpindi. This Experimental Station is situated in the northwest part of the Punjab, in a submountainous tract. The soil of this farm is deficient in available phosphates and shows a remarkable response to the application of phosphatic manures, especially superphosphate. Representative samples of wheat were collected from plots fertilized as shown in Table I.

There were eight treatments, each in quadruplicate. The size of each plot was 1/40th of an acre. Representative samples were collected separately from all of these plots, and were freed from dust and foreign matter. The required quantities were ground to a uniformly fine meal, thoroughly mixed to obtain a homogeneous sample, and

TABLE I
AMOUNTS OF FERTILIZER APPLIED PER ACRE

No.	Fertilizer treatment	Nitrogen as N ₂	Phosphorus as P ₂ O ₅	Green manure
		lb.	lb.	tons
1	Control (No manure)	0.0	0.0	0.0
2	Green manure (<i>Cajanus indicus</i>)	0.0	0.0	5.0
3	Potassium nitrate	60.0	0.0	0.0
4	Potassium nitrate, green manure	60.0	0.0	5.0
5	Superphosphate	0.0	25.0	0.0
6	Superphosphate, green manure	0.0	25.0	5.0
7	Superphosphate, ammonium sulfate	60.0	25.0	0.0
8	Superphosphate, ammonium sulfate, green manure	60.0	25.0	5.0

preserved in airtight bottles. The samples from each plot were analyzed for various chemical constituents.

Total phosphorus was determined by the standard A.O.A.C. method of estimating phosphorus volumetrically. Phytic phosphorus was determined by the procedure of McCance and Widdowson (12) with the modification suggested by Snook (21). The modification consisted in using 2 ml. of concentrated sulfuric acid instead of a mixture of sulfuric acid and perchloric acid for the wet digestion of sodium phytate. Nonphytic phosphorus was obtained by subtracting the amount of phytic phosphorus from the total phosphorus.

Calcium was estimated in accordance with the A.O.A.C. volumetric method involving precipitation as calcium oxalate and titration with standard potassium permanganate solution.

In the *in vivo* studies, the technique based on the "balance sheet" method as used by Henry and Kon (9) and Giri (5) was adopted. Five groups of young albino rats, weighing 60 to 70 g., each group having two males and two females, were placed in metabolism cages. The basic portion of the diet fed to the animals was composed of the following parts: dried egg white powder, 14; salt mixture (phosphorus and calcium-free), 3; powdered sugar, 10; coconut oil, 5; and cod liver oil, 3. In addition pure thiamine (3 γ) was given daily along with the food. The cereal constituted 75% of the diet. The rats were fed with weighed quantities of the diets, which were in excess of the normal requirements of the different groups. The residues were collected daily and dried in an electric air oven at 100°C. After a preliminary period of 3 days, feces and urine were collected daily for a period of 14 days. The urine of each group was mixed together, carefully concentrated on a water bath, dried on a sand bath, and the phosphorus determined volumetrically after ashing a known amount. Similarly, dry feces voided by each group were mixed, powdered, and passed through a fine mesh sieve. A weighed amount, 10–12 g., was ashed in a platinum dish and the phosphorus in the ash determined volumetrically.

Results and Discussion

Effect of Fertilization on Yield. The mean yields of wheat grain obtained from plots given different fertilizer treatments are shown in Table II. The application of various fertilizers to plots sown with wheat appreciably enhanced the yield of grain in all cases. There is a marked increase (38–48%) in the yield of grain of plots receiving superphosphate in various combinations as compared with the yield of grain from control plots. The addition of green manure along with the artificial fertilizers did not materially increase the yield above that

TABLE II
EFFECT OF FERTILIZATION ON YIELD OF WHEAT

No.	Fertilizer treatment	Mean yield per acre	Mean yield of main treatments, per acre	Mean yield of main treatments, as per cent of control	Increase or decrease due to green manuring
1	Control (No manure)	lb. 664.0	lb. 700.0	% 100.0	% +10.8
2	Green manure	736.0			
3	Potassium nitrate	873.6	831.6	118.8	- 9.6
4	Potassium nitrate, green manure	789.6			
5	Superphosphate	992.0	1038.0	148.2	+ 9.3
6	Superphosphate, green manure	1084.0			
7	Superphosphate, ammonium sulfate	928.0	970.0	138.2	+ 9.1
8	Superphosphate, ammonium sulfate, green manure	1012.0			

obtained with artificial fertilizers alone. While the yield was increased by about 9.1 to 10.8% by green manure in plots receiving fertilizer treatment Nos. 2, 6, and 8, they were lower by about 9.6% in the case of potassium nitrate applied with green manure. It may be that in the bacterial decomposition of green manure some of the nitrates might have been consumed.

TABLE III
EFFECT OF FERTILIZATION ON TOTAL PHOSPHORUS, PHYTIC PHOSPHORUS, NONPHYTIC PHOSPHORUS, AND CALCIUM CONTENT OF WHEAT
(Analytical data expressed on moisture-free basis)

No.	Fertilizer treatment	Phosphorus per 100 g.			Non-phytic ¹ P	Phytic ¹ P	Calcium per 100 g.
		Total	Phytic	Non-phytic			
		mg.	mg.	mg.	%	%	mg.
1	Control (No manure)	243.5	170.8	72.7	29.9	70.1	70.4
2	Green manure	272.2	179.6	92.6	34.0	66.0	70.5
3	Potassium nitrate	243.6	178.5	65.1	26.7	73.3	71.5
4	Potassium nitrate, green manure	263.3	180.7	82.6	31.4	68.6	72.0
5	Superphosphate	276.7	191.7	85.0	30.7	69.3	71.2
6	Superphosphate, green manure	316.3	199.4	116.9	37.0	63.0	73.8
7	Superphosphate, ammonium sulfate	299.8	222.6	77.2	25.7	74.3	82.8
8	Superphosphate, ammonium sulfate, green manure	264.5	202.7	61.8	23.4	76.6	74.9

¹ Phytic and nonphytic phosphorus as per cent of total phosphorus.

Effect of Fertilization on Chemical Composition of Grain. The results of chemical analyses of the wheats are given in Table III. The total phosphorus and phytic phosphorus content of the grain obtained from plots to which superphosphate was applied in various combinations (treatment Nos. 5, 6 and 7, 8) was higher than the corresponding values of the grain obtained from control and potassium nitrate-treated plots considered together. The mean total and phytic phosphorus of wheat obtained from plots receiving superphosphate (treatment Nos. 5, 6 and 7, 8) was 289.3 and 204.1 mg./100 g. as compared with 255.6 and 177.4 mg./100 g. for the grain from plots which did not receive superphosphate (treatment Nos. 1, 2 and 3, 4). The calcium content of wheat grain obtained from plots receiving phosphatic treatments, with the exception of treatment No. 5, was also higher than that of the grain from nonphosphatic treatments. Total phosphorus and nonphytic phosphorus content of the grain which received superphosphate and green manure was the highest, i.e., 316.3 and 116.9 mg./100 g., respectively, while the grain obtained from plots treated with superphosphate in conjunction with ammonium sulfate showed the highest content of phytic phosphorus and calcium, i.e., 222.6 and 82.8 mg./100 g., respectively.

The application of various fertilizers with green manure, with the exception of treatment No. 8, produced grain with comparatively high contents of calcium and total phosphorus (both phytic and nonphytic). The combination of green manure with superphosphate and ammonium sulfate, however, decreased these constituents in the grain. The readily available nonphytic phosphorus constituted 23.4 to 37.0% of the total phosphorus of the grain. The percentage proportion of phytic phosphorus to total phosphorus was the lowest (63.0%) in the grain from plots treated with superphosphate and green manure, and was the highest (76.6%) in the grain from treatment No. 8. From these results it can be tentatively concluded that combination of ammonium sulfate with superphosphate is conducive to the formation of more phytic phosphorus in the grain.

Relationship between the Calcium and Phytic Phosphorus Content of the Grain. In view of the possible antagonizing action of phytic acid on the absorption of calcium in the system, it was of interest to know if there was any association between the calcium and phytic phosphorus contents of the various samples. The coefficient of correlation between these variables was calculated from the results of 32 individual samples obtained from the various fertilizer treatments and found to be $r = +0.74$, a value which was highly significant. The relative nutritive value with respect to calcium and phytic phosphorus may, therefore, not have been much influenced by the different

manurial treatments, since an increase in the phytic phosphorus content of the grain is usually accompanied by a corresponding increase in the calcium content.

Effect of Fertilization on the Biological Availability of Phosphorus. The nonphytic phosphorus content of the wheat samples may be regarded as more indicative of the nutritive value with respect to available phosphorus. As not more than 23.4 to 37.0% of the total phosphorus content of the wheats appeared to be in the available form, it was considered desirable to determine the relation between available phosphorus content of the grain and the results of biological assays for a few typical composite samples of wheat representing different manurial treatments. The results for the percentage of biologically available phosphorus on the basis of total phosphorus for the total period of the experiment are given in Table IV.

TABLE IV
TOTAL INTAKE, EXCRETION, AND BALANCE OF PHOSPHORUS RETAINED BY RATS
FED ON VARIOUS DIETS CONTAINING 75% OF WHEAT GROWN ON
PLOTS WHICH HAD RECEIVED DIFFERENT FERTILIZERS

Rat group ¹ No.	Fertilizer treatment	In-take	Excretion			Phosphorus retained	Availability ²	Ratio ³ of phytic to nonphytic, P
			Urinary	Fecal	Total			
		mg.	mg.	mg.	mg.	mg.	%	
I	Control (no manure)	223.4	25.1	70.8	95.9	127.5	57	2.34
II	Superphosphate	303.5	25.3	85.8	111.1	192.4	63	2.25
III	Superphosphate, green manure	295.2	23.5	78.7	102.2	193.0	65	1.70
IV	Superphosphate, ammonium sulfate	250.1	21.9	82.5	104.4	145.7	58	2.88
V	Superphosphate, ammonium sulfate, green manure	237.6	30.5	78.6	109.1	128.5	54	3.28

¹ There was not much difference in the growth of the various groups.

² Availability as percentage of total phosphorus retained by the animals.

³ Calculated by dividing the values of phytic phosphorus by the corresponding values of nonphytic phosphorus of the parent wheat samples (Table III).

The biological availability of the phosphorus of the different wheat samples ranged from 54 to 65%. The former figure (54%) was obtained for the wheat samples obtained from plots treated with superphosphate, ammonium sulfate, and green manure, while the latter (65%) for the wheat samples obtained from plots treated with superphosphate and green manure. On the basis of the nonphytic phosphorus content, as revealed by chemical analysis, the available phosphorus should constitute only 23.4 to 37.0% of the total phosphorus. Collating this with the values obtained for biologically available phosphorus it is evident that roughly 27 to 32% of the phytic phos-

phorus of the wheat samples was also available to the rats. The exact nature of the different forms in which phosphorus exists in the cereals is still obscure, but certainly a major portion of it exists as phytin, a calcium and magnesium salt of phytic acid. This compound is susceptible to enzymic decomposition. Patwardhan (19) showed that an enzyme capable of hydrolyzing phytin is present in the intestines of the albino rats. This enzyme may bring about a partial conversion of the organic phosphorus into an inorganic form, thus rendering a part of it available to the system. McCance and Widdowson (12) determined the actual amount of phytic phosphorus in the feces after adding phytin to the diet of human beings. They found that 20 to 60% of the phytin was excreted unchanged in the feces and suggested that much of the remaining phosphorus may also have remained unabsorbed and excreted through the feces in some other form. On the other hand, Anderson (1) isolated crystalline inositol monophosphate from an organic phosphorus fraction of wheat bran, which had resisted breakdown into inorganic phosphorus even after preserving it for two years.

It may also be possible that phytic phosphorus is not completely digested in the stomach and some of it passed out undigested. This explanation appears to be plausible, since in the laboratory 0.5 *N* hydrochloric acid solution is used to extract this form of phosphorus from the test material, whereas the strength of this acid in normal human gastric juice is approximately 0.1 *N*. Acid of the latter strength can extract only small amounts of the phytic phosphorus. It is, however, possible that the enzyme pepsin, which is also present in the gastric juice, may exercise some influence on its digestion in the stomach. The latter point was investigated by carrying out an *in vitro* digestion of a sample of wheat meal with 0.1 *N* hydrochloric acid solution alone and with the addition of pepsin. The extraction of phytic phosphorus with 0.1 *N* hydrochloric acid was 56.3%, but with a combination of pepsin and 0.1 *N* hydrochloric acid it was 86.8%. It is likely that extraction under natural conditions may be still more efficient. In the light of this observation, the view that phytic phosphorus may not be extracted completely from the grain complex in the stomach is not correct. However, the phytic phosphorus may also be regarded as present in some loose combination with the proteins of the grain, as a fair amount of it was discovered to have been released on enzymic digestion.

The differences in the percentage of biologically available phosphorus among the tested samples seem to bear some relation to their phytic and nonphytic phosphorus content. On comparing the figures for biologically available phosphorus and the ratio of phytic phosphorus to nonphytic phosphorus (Table IV) there appears to be

negative correlation between the two. The maximum value for biologically available phosphorus was obtained with wheat which had received superphosphate and green manure. Addition of green manure to the plots receiving superphosphate and ammonium sulfate decreased the total and nonphytic phosphorus content of the grain (Table III) as well as the percentage of biologically available phosphorus, which was the lowest in case of this treatment.

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IMPORTANCE OF MALT EXTRACTION IN THE DETERMINATION OF AMYLASE ACTIVITIES ¹

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ABSTRACT

Two laboratory methods of extracting malt which will give the highest or maximum alpha-amylase values are described. The regular method involves extraction with 0.4% sodium bicarbonate solution at 30°C. for two hours; in the rapid method the extraction is conducted by stirring the malt with this solution at 35°C. in a Waring Blendor for 10 minutes. The maximum alpha-amylase values obtained by these methods are more representative of those experienced with malts in the brewing, distilling, and baking industries than the activities obtained with distilled water extracts. The temperature coefficient of dextrinization (Q_{10} , 30°/20°C.), was found to be 1.9 when the temperature effect on the color comparison is eliminated; as this coefficient is constant for different malts and extraction conditions, the alpha-dextrinizing activity can be determined either at 20°C. or 30°C. and the results calculated for both.

Although diastatic power determinations on distilled water extracts of barley malts do not reflect the activities realized commercially as well as extracts prepared by the regular method, they are sufficiently accurate for comparative purposes. Wheat malts may contain large quantities of salt-soluble beta-amylase, and hence reducing-sugar determinations on distilled water extracts fail to reveal their potential saccharifying activity when used in the distilling and baking industries.

In a recent study the authors discussed how the extraction of barley and wheat malts with dilute salt solutions results in extracts with markedly higher alpha-amylase activity and also higher total saccharifying power than extracts made with distilled water (5). The most suitable conditions for obtaining extracts of the highest amylase activity designated as maximum alpha-amylase activity were extraction with 0.05 to 0.2 *N* solutions of sodium acetate, bicarbonate, nitrate, or secondary phosphate at 30°C. Employing these conditions, the maximum alpha-amylase values of the extracts were relatively insensitive to variations in salt concentration within the foregoing concentrations, to extraction temperatures between 30° and 45°C., and to increasing the extraction time beyond the minimum necessary for complete dispersion.

In the present paper, two laboratory methods are described for the routine determination of maximum alpha-amylase activity in malt.

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Methods for Routine Determination of Maximum Alpha-Amylase Activity

In principle, the determination of maximum alpha-amylase activity involves the application of modified extraction procedures to the dextrinization methods of Sandstedt, Kneen, and Blish (14) and Olson, Evans, and Dickson (9, 10).

Choice of Salt. Sodium bicarbonate was selected as an appropriate solvent since 0.2 to 0.4% solutions will give maximum and consistent alpha-amylase values while 0.5 to 2.0% solutions of sodium acetate, biphosphate, and nitrate are required to obtain equivalent but less consistent activities.

Temperature and Duration of Extraction. The extraction may be conducted at any temperature between 30° and 45°C. provided the extraction time is adequate. The time necessary to secure maximum extraction is shortened when the temperature is raised (or the infusion is agitated) (5). This is illustrated in Fig. 1 in which the percentages of maximum alpha-amylase activity of a typical brewers' barley malt are plotted against time of extraction with 0.4% sodium bicarbonate solution at 20°, 30°, and 40°C. respectively. Maximum activities were obtained in about 1 hour at 30°C., and in 30 to 40 minutes at 40°C.

When the malt infusion was agitated in a Waring Blender, maxi-

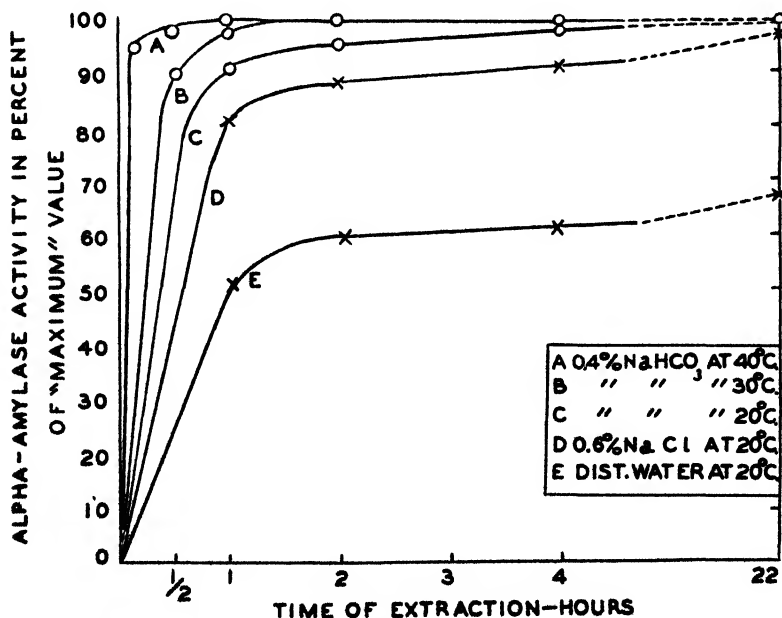


Fig. 1. Effect of time of extraction on alpha-amylase activity in the extract of an average brewers' barley malt. Comparison of extractions with 0.4% NaHCO₃ solutions at 20°, 30°, and 40°C., 0.6% NaCl solution at 20°C., and distilled water at 20°C.

imum values were obtained in 10 minutes at 35° to 40°C. Little or no destruction of alpha-amylase occurs if the pH is maintained between 5.0 to 8.0.

Dextrinization. Olson, Lowry, and Dickson (11) failed to obtain a constant relation for different malts between the alpha-amylase activities of distilled water extracts at 20° and 30°C. and determined at 20° and 30°C. respectively. The variability in the ratio between the activities for these temperatures was not reduced by extracting with 0.5% sodium chloride solution, although the average ratio was lower. The lack of a constant relation between the activities obtained at 30° and 20°C. was not due to the dextrinization step of the test since dextrinizations conducted at 20° and 30°C., for a series of malts extracted with distilled water at 20°C., gave a temperature coefficient (Q_{10} , 30°/20°) for dextrinization of 2.13 which was relatively constant for the different malts. In this study the dextrin color comparisons were made at the temperatures at which the dextrinizations were carried out so that the temperature coefficient includes any effect of the 10° difference in temperature on the dextrin-iodine color comparison.

Similar studies were conducted by the authors in which seven malts of varying activity were extracted with distilled water and with a variety of different salt solutions (0.5 to 2.0 *N*) at temperatures of 20°, 30°, and 40°C. for different periods of time. Dextrinization measurements were conducted with each extract at 20°C. and 30°C., but the color comparisons were all made at 20°C. The temperature coefficients in all these trials were within $\pm 4\%$ of 1.88; as the maximum deviation was well within the limits of experimental error, the coefficient is considered to be constant and independent of the nature of the malt, the extraction medium, or of the time and temperature of the extraction. To determine whether the differences between this coefficient and that of 2.13 found by Olson *et al.* could be ascribed to the effect of temperature on color comparisons noted by Redfern (12), samples of 10 malts were extracted with different solutions for varying times and temperatures. The dextrinizations were conducted at 30°C., but the color comparisons were made at both 30° and 20°C. Alpha-amylase activities calculated from the end point at 30°C. divided by those at 20°C. yielded a mean factor of 1.15. Multiplying the coefficient 1.88 by this factor gives a value of 2.16 which is in good agreement with the Q_{10} of 2.13 found by Olson *et al.* The temperature coefficient of the dextrinization reaction proper is therefore 1.88. The temperature coefficient of saccharification from the data for maltose formation at 30°C. and 20°C. has also been determined under conditions paralleling those reported for dextrinization.

It was found to fall between 1.80 to 1.84, so that the temperature coefficients for both phases of starch hydrolysis are of the same order of magnitude.

Methods. The studies which have been described provide a basis for the establishment of convenient routine methods for the determination of maximum alpha-amylase activity of malts. The use of suitable salt solutions eliminates the influence of temperature on the quantity of alpha-amylase extracted. Since the dextrinization values at 20° and 30°C. bear a constant relation to each other, the test can be conducted at either temperature according to convenience. Two methods, a regular and a rapid method, are described.

REGULAR METHOD: Twenty-five grams of finely ground malt are extracted with 500 ml. of 0.4% sodium bicarbonate solution at 30°C. for 2 hours and filtered at room temperature. Ten milliliters of the clear filtrate are diluted to 100 ml. with distilled water, adjusted to 20°C., and the dextrinization time determined on a 10-ml. aliquot as described by Olson, Evans, and Dickson (9, 10). The color comparison may be made in accordance with this method or with the glass end point color standard of Redfern (12).

RAPID METHOD: Five grams of ground dry malt, or 10 g. of whole wet malt are added to the glass or aluminum bowl of a Waring Blendor containing 500 ml. of 0.4% sodium bicarbonate solution which has been brought to about 35°C. The mixer is operated for 10 minutes at low speed for ground malt, and at high speed for whole malt. The malt infusion is then filtered through a fluted filter sufficiently large to take the entire charge, the first 100 to 200 ml. being returned to the filter. A portion of the clear filtrate is brought to 20°C. and the dextrinization time determined on an aliquot as described by Olson, Evans, and Dickson (9, 10). With dry malts of moderate activity the determination is made with a 10-ml. aliquot (0.1 g. malt); with highly active malts a 5-ml. aliquot is taken. For malts of exceptionally low activity, double the usual quantities are extracted. The moisture content of wet malts is determined at the time the samples are weighed for extraction.

This method does not require a water bath for the extraction and the entire determination can be completed in less than an hour. Comparison of the values with those obtained by the regular methods is shown in Table I. For dry malts the results are in satisfactory agreement,⁴ but somewhat greater differences were obtained between the values for fresh malts analyzed by the rapid method and those for the corresponding dried malts by the regular method; this appears to be

⁴ Similar agreement of results was found when the two methods were compared using suitable salts other than sodium bicarbonate.

TABLE I
COMPARISON OF ALPHA-AMYLASE VALUES OBTAINED
BY REGULAR AND RAPID METHODS

Sample number	Alpha-amylase activity (20°C. dextrinizing units)	
	Regular method	Rapid method
Brewers' barley malt		
1	33	33
2	33	33
3	33	33
4	29	28
5	31	32
6	29	28
7	31	32
Distillers' barley malt		
8	44	44
9	40	40
10	30	30
Malted wheat flour		
11	18	18
Green barley malt		
12	35 ¹	40 ²
13	47 ³	49 ²

¹ Vacuum dried.

² Wet.

³ Kiln dried.

due to changes in activity occurring during dehydration rather than to the methods.

Determination of Saccharifying Activity; Diastatic Power

The diastatic power of malt reflects the composite saccharifying activities of beta- and alpha-amylase and perhaps other enzymes. When Kjeldahl in 1879 (6) and Lintner in 1885 and 1908 (7, 8) introduced this assay, they infused the malt at room temperature for 6 hours and assumed that the extraction of diastase was complete. The lack of agreement in the results obtained in different laboratories led to extensive collaborative work to develop an acceptable standardized procedure (1, 2, 3). Variations in the conditions of extraction were found to be one source of error (13). Strict adherence to the prescribed temperature, time, and concentration is necessary to assure reproducible results, especially since the conventional conditions (malt ratio, 1:20; 20°C.; and 2.0 or sometimes 2:5 hours) do not give complete extraction of the amylases. The authors have shown that the extraction of alpha-amylase from both barley and wheat malt is markedly influenced by salts and by temperature and this must in turn affect the diastatic power. The extraction of beta-amylase from barley malt is little affected by these variations in the conditions of extraction, but this does not appear to be true for wheat malts. It is

therefore necessary to consider separately the most appropriate conditions for determining the diastatic power of barley and wheat malt.

Barley Malt. The extraction conditions shown to give maximum alpha-amylase activities are approximately those used in technical mashing operations. The salts most suitable for this purpose slightly stimulate beta-amylase at 20° and 30°C. in solution, but apparently are not important in its release (5). Extraction at 40°C. and for 1 hour results in some destructive heat effect which about compensates that stimulation. It may be assumed that conventional malt extraction at 20°C. with distilled water yields almost the same amount of beta-amylase activity as is obtained in industrial operations, and that the actual increase in diastatic power is practically that of the increased alpha-saccharifying activity. Table II illustrates these effects on commercial barley malts.⁵

The increase in alpha-amylase activity due to the use of salts and higher extraction temperatures raises the diastatic power above that determined in the conventional manner by extracting with distilled water at 20°C. The two values for this increase under sections A and B of the table represent the range of variation which may occur with brewers' and distillers' malts under practical conditions; the smaller figure is for malts of high diastatic power with a relatively small increase in alpha-amylase activity; while the larger figure applies to the reverse condition. Although the ratio of the alpha-amylase activities for salt extracts as compared with water extracts varies considerably, the variations in the effect upon diastatic power are well within the commonly accepted margin of experimental error. The conventional diastatic power determination, therefore, can serve for the comparative evaluations of malts of the same general type. If values more strictly conforming to technical mashing conditions are desired, the extraction procedure described as the regular method may be employed, or the extraction can be made with industrial water at 40°C. for 1 hour. The rapid method is not suitable for this purpose because the vigorous stirring leads to a decrease in beta-amylase activity.

Wheat Malt. The influence of extraction conditions on the diastatic power of the wheat malts shown in Table II is strikingly different from that found with barley malts because of the presence of large quantities of salt-soluble beta-amylase in the former. The increase in beta-amylase activity due to extraction with salts and to increasing the temperature or duration of extraction so markedly influenced the diastatic power that it greatly overshadowed the higher alpha-saccharifying activity.

⁵ Beta-amylase activity was calculated as the difference between the maltose equivalents of the total saccharifying power and of the alpha-dextrinizing activity. We used the mean values of these equivalents as published by Olson, Evans, and Dickson (9) and by Ehrnst and Lucht (4) which are somewhat divergent at higher alpha dextrinizing values.

TABLE II
INFLUENCE OF SALT AND TEMPERATURE DURING EXTRACTION ON
DIASTATIC POWER AND BETA-AMYLASE

Extracting medium	Diastatic power in % of conventional maltose units			Effect upon D.P. of alpha-amylase in- crease in % of con- ventional D.P.			Beta-amylase in % of distilled water- values at 20°C.			
	Temp. of extraction Hours of extraction	20° 2	30° 2	40° 1	20° 2	30° 2	40° 1	20° 2	30° 2	40° 1
A. COMMERCIAL BREWERS' BARLEY MALTS ¹ (100°-125° L., 30-35 Maximum alpha, 20° Dextrinizing Units)										
Distilled water	100	101	102	0	3-4	4-5	100	98	96	
0.6% NaCl	108	108	108	7-8	7-8	7-8	101	101	101	
0.4% NaHCO ₃	111	116	110	7-10	7-10	7-10	104	108	100	
B. COMMERCIAL DISTILLERS' BARLEY MALTS ² (180°-200° L., 40-45 Maximum alpha, 20° Dextrinizing Units)										
Distilled water	100	104	103	0	2-3	3-4	100	102	99	
0.6% NaCl	104	108	105	4-6	5-7	6-8	100	103	99	
0.4% NaHCO ₃	105	108	107	5-7	6-9	6-9	100	101	100	
C. WHEAT MALT (106° L.; 33 Maximum alpha, 20° Dextrinizing Units)										
Distilled water	100	105	107	0	2	3	100	104	105	
0.6% NaCl	146	155	164	4	4	3	155	167	167	
0.4% NaHCO ₃	147	153	149	3	4	3	158	160	160	
D. COMMERCIAL MALTED WHEAT FLOUR (57° L., 18 Maximum alpha, 20° Dextrinizing Units)										
Distilled water	100	123	137	0	5	3	100	122	139	
0.6% NaCl	160	170	—	3	10	— ^u	169	174	—	
0.4% NaHCO ₃	—	165	—	—	10	—	—	167	—	

¹ The analytical values are the means for two brewers' malts, dried at final temperatures of about 72°C.

² The results are the means for three distillers' malts dried at about 50°C.

These limited results suggest that distilled water extracts of wheat malt are unsatisfactory for the reliable determination of amylase activity. Neither the conventional diastatic power test nor the alpha-amylase determination on such extracts may reflect the enzymic activities of wheat malt for use in baking or in the distilling industry. Further studies are necessary to develop a suitable extraction procedure.

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BREEDING FOR NIACIN CONTENT IN A SORGHUM CROSS, WESTLAND X CODY¹

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ABSTRACT

The niacin content of grain from F₁, F₂, and F₃ plants of a Westland X Cody cross is presented.

Westland ranged from 43.0 to 49.1 μg . niacin per gram and Cody ranged from 66.9 to 72.9 μg . per gram. The grain from the F₁ plant contained 46.3 μg . per gram. The grain from the F₂ plants contained niacin in concentrations ranging from 37.8 to 103.6 μg . per gram. Grain from one F₃ plant had a niacin content of 124 μg . per gram, the highest value thus far found for sorghum.

Niacin content appears to be an inherent varietal characteristic. The development of sorghum varieties with high niacin content appears to be possible.

The need for dietary niacin by certain classes of animals including swine, poultry, and human beings has been well established, although

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the physiology and biochemistry of this vitamin remain obscure. Recent tests from numerous laboratories have indicated that the amount of dietary niacin required may depend upon the quality of the protein in the diet. Increased niacin requirements have been noted with rations compounded with certain proteins, especially those deficient in tryptophane. In view of the general deficiency of this amino acid in the proteins found in cereal grains and of the biological interrelationship between niacin and tryptophane, the niacin content of grains apparently becomes an important characteristic to be considered in formulating rations.

Little is known concerning factors influencing the vitamin content of plant materials. Several factors undoubtedly play important roles, of which climatic conditions, soil types, and fertilizer as affecting nutrition of the plant have been studied in some instances. The literature pertaining to the influence of some of these factors has been reviewed by Somers and Beeson (8). In general, however, the inheritance of vitamin content has received little attention.

A few reports are available which suggest that perhaps the content of B-complex vitamins in cereals may be altered by breeding new varieties. Burkholder, McVeigh, and Moyer (2) presented niacin analyses of 46 strains of corn which averaged 34.6 $\mu\text{g.}$, but ranged from 18.2 to 62.1 $\mu\text{g.}$ of niacin per gram on an air-dry basis. Sweetcorn varieties generally contained more niacin than dent varieties, and popcorn varieties averaged lowest in niacin. Barton-Wright (1) found that sweetcorn contained nearly twice as much niacin as the flint corns he analyzed (31.0 and 15.6 $\mu\text{g.}$ per gram, respectively). Later, Mather and Barton-Wright (5) analyzed sugary and starchy kernels from the same open-pollinated ears. Presumably the starchy kernels were developed following pollination with starchy types and the sugary kernels following pollination by sugary types. In five such sets of samples analyzed, the sugary grain averaged 26.2 $\mu\text{g.}$ and the starchy grain 14.0 $\mu\text{g.}$ niacin per gram, with small individual differences attributable to strain characteristics. Richey and Dawson (6) concluded that corn hybrids with niacin concentrations as high as 50 $\mu\text{g.}$ per gram probably could be developed. They suggested that two modes of niacin inheritance may function in corn. One involves the joint action of many genes of small individual effects with dominance lacking, and the second mode, following the suggestion of Mather and Barton-Wright (5), may involve *Su su* alleles, with *Su* dominant for lower niacin concentration.

Hunt, Ditzler, and Bethke (3) reported the niacin and pantothenic acid content of nine double-cross corn hybrids grown at five experiment stations in three successive years. Variations were found for

all crop years, and all locations, but the greatest variation in niacin content was ascribed to varietal influences. In Cody sorghum, on the other hand, Tanner, Pfeiffer, and Curtis (9) found that the niacin content in grain grown at Hays, Kansas, from 1942 through 1945 were 72.1, 72.9, 70.5, and 71.8 $\mu\text{g.}$ per gram respectively. This would indicate high stability in the niacin level from year to year even though the seasons were widely different. Similar stability was obtained for several other sorghum varieties grown for a shorter period.

The data available on grain sorghums suggest that niacin content is a varietal characteristic that may be altered by breeding practices. Knox *et al.* (4) observed wide differences in the niacin content of 29 grain sorghum varieties grown under comparable conditions. Similar data were obtained by Tanner, Pfeiffer, and Curtis (9) for 48 commercial and experimental varieties from different sources and locations. The niacin content ranged from 27.7 to 91.9 $\mu\text{g.}$ per gram on a moisture-free basis. Some strains developed by hybridization carried niacin levels much higher than either of the parental varieties. For instance, four Cody \times Wonder Club strains grown at Hays, Kansas, in 1945 contained 76.6 to 91.9 $\mu\text{g.}$ of niacin per gram; three of these were significantly richer in niacin than Cody, the high-niacin parent variety.

Since the data suggested that new lines of grain sorghum could be developed that would be rich in this vitamin, its inheritance in one series of samples, representing a cross of Westland \times Cody, was studied. Westland is a commercial variety relatively low in niacin and Cody is a commercial variety relatively rich in niacin.

Methods and Materials

Niacin determinations were made by the microbiological method of Snell and Wright (7) after hydrolysis of samples in 1 *N* sulfuric acid for 30 minutes at 120°C. Hydrolyzates were adjusted to pH 6.8, filtered, and assayed in the conventional manner. All results are expressed on a moisture-free basis.

The seed samples were from individual F_2 plants of a Westland \times Cody cross grown at Hays, Kansas, in 1945. After these strains had been analyzed, remnant seed was planted the following year, and random samples of the seed from F_3 plants were analyzed to check the stability of these new lines. Care was taken to include F_3 plants from parental lines ranging from low to high in niacin content.

Results

Seed from the specific plants of Westland and Cody used in this cross was not available, but pure lines of both varieties were analyzed

for two and five years, respectively. In Westland the niacin content ranged from 43.0 to 49.1 $\mu\text{g.}$ per gram; for Cody the range was from 66.9 to 72.9 $\mu\text{g.}$ per gram with only one sample, that of the 1946 crop, having less than 70 $\mu\text{g.}$ per gram. The amount of niacin in Westland appeared to be stabilized at a relatively low level compared to Cody. The F_1 generation of the cross, grown at the Hays station in 1944 and assayed in 1947, contained 46.3 $\mu\text{g.}$ of niacin per gram.

The F_2 generation plants were grown in 1945. Seed from 335 plants was harvested and analyzed. The niacin content ranged from a low of 37.8 to a high of 103.6 $\mu\text{g.}$ per gram. Two samples of Cody produced the same crop year averaged 69.4 $\mu\text{g.}$ per gram. The data for the 335 plants were grouped into classes each with a spread of 5 $\mu\text{g.}$ per gram. Only seven plants had niacin content as low as or lower than the Westland variety, the low parent, while 127 plants contained niacin concentrations equal to or greater than Cody, the high-niacin parent, and 201 plants were intermediate between the parental varieties. In this group, the majority of plants were above the midpoint between the parents. This distribution of samples is shown graphically in Fig. 1.

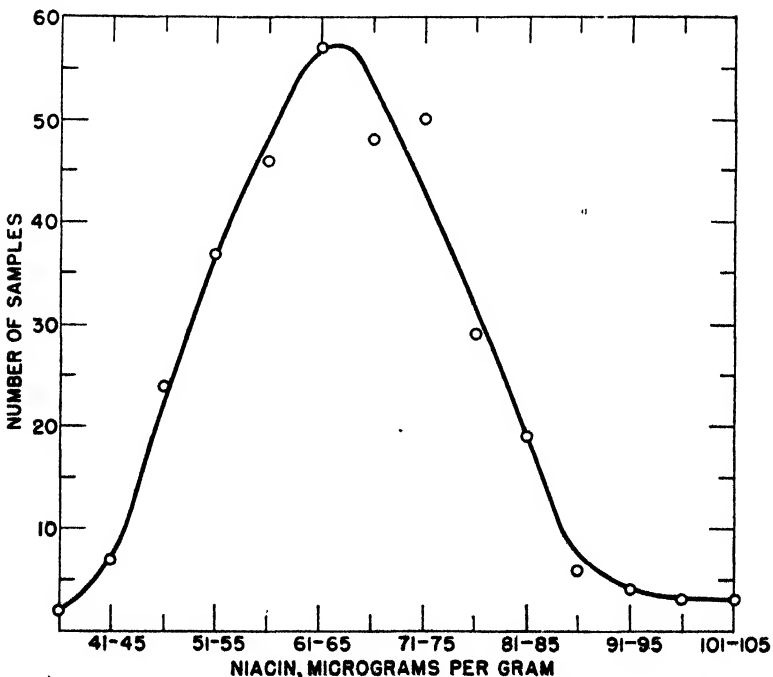


Fig. 1. Distribution of niacin in seed from F_2 plants of Westland \times Cody cross.

No attempt was made to assay all F_3 heads for niacin because of the large number of samples involved. However, five groups were analyzed involving bulk seed and two or three plants from rows selected to include those produced from randomly selected F_2 plants of high, intermediate, and low niacin content. The niacin analyses of this group of samples are shown in Table I.

TABLE I
NIACIN CONTENT OF BULK SEED AND SEED FROM SEPARATE PLANT
SELECTIONS OF F_3 GENERATION WESTLAND \times CODY

Niacin content in	Groups				
	1	2	3	4	5
F_2 plant, 1945	102.3	63.1	$\mu\text{g./g.}$ 46.9	43.3	39.3
F_3 plant, 1946 Plant 1	91.4	48.9	42.2	53.3	92.2
Plant 2	42.0	50.1	48.2	56.5	95.1
Plant 3	75.0	60.2	—	—	57.3
Bulk seed	84.4	51.3	54.7	79.3	45.3

In general the bulk seed produced on high-niacin rows tended to have a high-niacin concentration and that on low-niacin rows tended to be low. Segregation, however, occurred in each group. In Series 4, the bulk seed from the F_3 plants was considerably richer in niacin than the seed from the F_2 plants.

These data suggested that further analyses of the seed from F_3 plants would be necessary to indicate the potential range of variation in niacin content that might be expected. Additional analyses were made of 157 F_3 heads grown on 55 head-rows. Table II summarizes the results obtained. In only one group did the grain in F_3 heads

TABLE II
RANGE OF NIACIN IN SEED FROM F_2 AND F_3 PLANTS FROM THE CROSS
WESTLAND \times CODY, HAYS, KANSAS, 1945 AND 1946

No. of F_2 plants from 1945 propa- gated to F_3 in 1946	Range of niacin in the F_2 plants	Average ni- acin content F_2 plants, 1945	No. of F_3 plants ana- lyzed, 1946	Range of niacin content of F_3 plants, 1946	Avg. niacin content F_3 plants, 1946
	$\mu\text{g./g.}$	$\mu\text{g./g.}$		$\mu\text{g./g.}$	$\mu\text{g./g.}$
5	38.0- 49.1	45.0	14	43.9- 74.5	59.4
19	50.9- 59.3	55.2	55	35.5-109.9	64.8
15	60.3- 69.8	63.8	43	53.7-115.9	75.8
7	70.8- 79.9	73.5	18	51.4-124.0	76.3
6	80.1- 81.6	80.7	18	69.9-106.3	92.2
3	90.6-102.0	94.6	9	68.0-105.6	88.2
55			157		

have an average niacin content below that of the F_2 heads. In one F_3 row a head containing 124.0 $\mu\text{g.}$ of niacin per gram was found, which is the highest niacin concentration yet found in this cereal. This is more than 50 $\mu\text{g.}$ per gram higher than the Cody variety, the initial high-niacin parent.

So far as could be determined, niacin content of sorghums was not associated with any observable plant character that could be used as a genetic tester. As is the case with many other quantitative characters, the inheritance of niacin probably is complex and results from the interaction of many factors.

While an analysis of only three heads per row is not sufficient to measure the behavior of F_3 rows, the data indicate that new lines of grain sorghum rich in niacin may be obtained. Other agronomic requirements also must be met, but if future research demonstrates the desirability of developing cereal varieties of greater nutritional value, it should be possible to increase the niacin content of grain sorghums materially by selection from suitable crosses.

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DETERMINATION OF REDUCED IRON IN VITAMIN ENRICHMENT MIXTURES¹

J. D. NEUSS and H. A. FREDIANI²

ABSTRACT

When iron is present as reduced iron (iron by hydrogen) in vitamin enrichment mixtures it may be determined readily by magnetic separation and direct weighing. Such a method provides a rapid and precise method for determining the added iron content and precludes the inclusion of other iron compounds that may be present in the other components of the mixture.

The official A.O.A.C. method (2) for determining iron in cereal foods, including bread, involves wet ashing of the sample, reduction to the ferrous state with hydroxylamine hydrochloride, subsequent color development with alpha-alpha dipyridyl or orthophenanthroline, and measurement in a suitable colorimeter using applicable calibration curves. In somewhat similar fashion the official A.A.C.C. method (1) for iron in flour involves ashing of sample, dissolution of residue, reduction with hydroquinone, and colorimetric measurement with dipyridyl. No official method has been published for this determination in vitamin enrichment mixtures, so that current practice appears to be to utilize suitable modifications of the method used for flour. When the iron in such a mixture is present in the metallic state in the form of reduced iron, a magnetic flotation method may be utilized for the physical separation of the iron from the other nonmagnetic components. The sample is placed in a beaker supported on one pole of a strong Alnico magnet. The major portion of the vitamins and starch is removed by flotation with methanol or denatured ethanol. Several washings with distilled water then dissolve the remaining riboflavin not removed by the initial flotation. The residual iron is washed with methanol to remove water and finally rinsed with ethyl ether to remove methanol. Air drying for several minutes suffices to yield the iron in weighable form.

Materials and Methods

Into a tared 100-ml. beaker accurately weigh 1.0000 g. of the well-mixed sample of the vitamin mixture. Place this beaker on one pole of a large Alnico magnet, similar to catalog No. 12-012, Fisher Scientific

¹ Manuscript received April 26, 1949.

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Co. Add 15-25 ml. of reagent grade methanol, simultaneously washing down the inside wall of the beaker. Agitate the sample vigorously for 15-30 seconds with a stirring rod bearing a rubber policeman. Keep the base of the beaker against the pole of the magnet in the following and all subsequent washings and decantations. Rinse and remove the stirring rod. Decant the methanol suspension and repeat the flotation of nonmagnetic constituents with a second portion (15-25 ml.) of alcohol. Follow this with four successive washes using distilled water. The third water wash should be only faintly yellow and the final water wash should be colorless. Wash again with two portions of methanol and complete the washing process with two portions of ethyl ether. Let the beaker and residue of iron stand for 2-3 minutes until iron particles are dry. Upon removal of the beaker from the magnet the iron particles will be free-flowing and loose if properly washed and dried. Weigh the beaker with iron and note weight, in milligrams, of iron recovered. Multiply this value by 28.35 to obtain the iron content of the sample in milligrams per ounce.

<i>Sample</i>	TYPICAL DATA	
	A_1	A_2
Wt. sample	1.0000	1.0000
Wt. iron recovered	0.1764	0.1775
Average wt. iron recovered	177 mg.	
Mg. iron per ounce	5020	

Results and Discussion

Replicate samples have been found to be easily reproducible to 1-2 mg. (1%). In a series of five determinations carried out on a uniform sample the average deviation was found to be 0.5%, with a maximum deviation of 1%. The iron recovered from a series of 15 determinations was assayed by standard methods and found to be 97.5% pure, indicating negligible contamination by other components of the original mixture.

The adequacy of air drying of the iron residue was tested by further drying at 110°C. The additional loss was generally negligible and in no case exceeded 0.5 mg.

In Table I are listed the results obtained on 12 different samples both by the magneto-gravimetric and by a volumetric method. In this comparison, because of the relatively high iron contents of the samples studied, it was not found feasible to use the colorimetric A.O.A.C. procedure. Excessive dilution or unreasonably small samples would have been necessary in order to obtain reasonable color intensities. In the volumetric method used, the sample was ignited in a muffle, the residue then dissolved in hydrochloric acid, and reduced by passage through a Jones reductor. The ferrous iron

TABLE I
DETERMINATION OF REDUCED IRON IN VITAMIN ENRICHMENT MIXTURES

Sample	Magneto-gravimetric method mg. Fe per oz.	Volumetric method mg. Fe per oz.	Difference mg.
1	4977	4950	+27
2	4946	4890	+56
3	4976	5020	-44
4	4828	4970	-142
5	5105	5040	+65
6	4863	4890	-27
7	5093	5030	+63
8	4989	4850	+139
9	4852	4960	-108
10	4829	4750	+79
11	4977	4950	+27
12	4946	4890	+56

thus formed was titrated with standard ceric sulfate solution. The recommendations of Edmonds and Birnbaum (3) and of Smith (4) were generally followed in this part of the procedure. The average relative difference between the two methods was found to be 1.5%.

Acknowledgment

Acknowledgment is made to Mr. Walter Fulmer and the staff of the Vitamin Testing Section for the results obtained by the Ceric Sulfate method.

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BOOK REVIEWS

Seed Crushing, Compound and Provender Milling. By H. Moore and A. S. Moore. Volume II. 238 pp. Northern Publishing Co., Liverpool, England. Price: 10/-.

The present work is an extension of Volume I, with much greater emphasis on the subjects which originally were treated only briefly, or not at all. The series has been prepared for use as texts in courses offered by the Seed Crushing, Compound and Provender Manufacturing Industries. Both books are divided into two sections, Part I, "Seed Crushing," and Part II, "Compound and Provender Milling." In both cases, the technology of various operations conducted in oil and feed mills is described in excellent detail, with drawings illustrating clearly the functioning of the various machines employed in all steps of manufacturing. The methods and equipment, however, are entirely English and in most cases are at least slightly

different from those known to oil and feed producers in the United States. In many instances, the design of equipment is vastly different from that of American counterparts, but the mechanical features of the English machines deserve careful consideration because of rather obvious advantages over some of the equipment used in this country.

Under "Seed Crushing," Volume II contains much more information than does Volume I with regard to continuous screw presses, solvent extraction, and the handling of oil and meal. Even so, the types of screw presses and solvent extractors used in this country are not described. To do so, and to describe in equal detail the many other types of such equipment used throughout the world, would necessitate tremendous expansion of the book into something more than a text prepared for a specific course of instruction.

The second volume differs from the first with respect to the descriptions of compound and provender milling also. In English terminology, "compound" is the equivalent of mixed feed in the United States, whereas "provender" refers to such items as mill feed, hominy feed, and the like. The new volume is particularly complete in its descriptions of conveyors and equipment for handling solid materials. Among the other subjects treated quite fully, and which were not covered in the earlier book, are grain storage, drying, and size reduction.

Volume I and Volume II of this series are complementary, i.e., Volume II is not simply a revision of Volume I, but a presentation of information that could not be included in the course for which Volume I was prepared as a text. Both books should prove of great value to anyone interested in the actual operating details of an oil mill or feed plant, even though much of the English equipment described might be quite unfamiliar to him. Equivalent texts describing American machinery and methods would be of enormous value to those on the operating level in American mills.

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Theory of the Stability of Lyophobic Colloids: The Interaction of Sol Particles Having an Electrical Double Layer. By E. J. W. Verwey and J. Th. G. Overbeek. Natuurkundig Laboratorium N. V. Philips Gloeilampenfabrieken, Eindhoven (Netherlands). XI + 205 pp. 54 figs. 16.5 × 24 cm. With the collaboration of K. Van Nes Elsevier Publishing Company, Inc., 215 Fourth Avenue, New York 3, N. Y. 1948. Price \$4.50.

The purpose of this book is to develop a quantitative theory of the stability of hydrophobic colloids and suspensions in terms of the present day concepts of the electrochemical double layer and of the London-van der Waals forces. The basic idea involved in this theory is that a repulsion force, in combination with an attraction force, determines the repulsion or attraction potential which will function between the particles of disperse phase and defines the conditions of stability, or lack of stability, which will exist. The repulsion force results from the mutual interaction of the diffuse double layers, while the attraction force is due to London-van der Waals forces.

Roughly a third of the book is devoted to a treatment of the modern concepts of the structure of the electrical double layer and the nature of van der Waals forces. This discussion serves to orient the reader for the ensuing treatment of the theory of stability which is developed. The remainder of the book treats this theory from the standpoints of interactions which will occur between two parallel flat plates, as one case, and between spherical colloidal particles, as a second case. Predictions based upon the theory are shown to agree well with many of the experimental observations of colloid chemistry. For example, a theoretical basis for the Schultz-Hardy effect of electrolytes upon the stability of lyophobic colloids is given which shows good agreement with experiment. The theoretical work on the stability of lyophobic systems by previous authors is discussed in an appendix to this book.

The authors have performed a great service to colloid chemistry in their logical development of a theory in explanation of this very complex phenomenon. While further elaboration of the theory involved will undoubtedly be required, the present

treatment constitutes a remarkable and stimulating advance in the understanding of the factors involved in the stability of hydrophobic colloids and suspensions.

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The Chemistry and Technology of Enzymes. By Henry Tauber. 550 pp. John Wiley & Sons, Inc., New York and Chapman & Hall, Ltd., London. 1949. Price \$7.50.

This book replaces two by the same author, namely *Enzyme Chemistry* (1937) and *Enzyme Technology* (1943) which have not gained wide usage as standard references for students. In general the structure of the earlier books has been retained but the contents have been rewritten and considerably enlarged.

In Part 1, 15 chapters are devoted to enzyme chemistry, and methods for the preparation of several enzymes are included. The initial definition and classification of enzymes is slightly confusing. For example, cytochrome is placed in the iron-porphyrin enzyme group together with the remark that "the cytochromes . . . are not enzymes."

In Part 2, 16 chapters are concerned with the methods used in the industrial production of enzymes and their uses, and with the production of industrially important organic compounds by fermentation. In the last chapter, microbiological methods for the estimation of vitamins and amino acids are discussed. Naturally, in any one book which aims at covering such a broad field as does this one, there is a limit to the amount of discussion on the various topics. However, the author has covered the important aspects of his subjects. While most cereal chemists will probably disagree with the author in his statements on the role of proteinases in breadmaking, they will find that this book contains much useful information and many references on matters of interest to them.

The chemical formulae, graphs, and photographs are very well presented and the occasional errors, such as "*Streptococcus aureus*" instead of "*Staphylococcus aureus*," are relatively unimportant.

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SUGGESTIONS TO AUTHORS

General. Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, all original drawings or photographs for figures, and one set of small photographic reproductions of figures.

Issues of *Cereal Chemistry* published after January 1, 1948, when some changes were made, are the most useful guides to acceptable arrangements and styling of papers.

Titles and Footnotes. Titles should be specific, but should be kept short by deleting unnecessary words. The institution from which the paper is submitted, author's connections, etc., are given in footnotes.

Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. Long introductory reviews should be avoided, especially when a recent review in a monograph or another paper can be cited instead. References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also.

Organization. The standard organization involves main sections for abstract, introduction, materials, methods, results, discussion, acknowledgments, and literature cited. Alternately, a series of separate studies is often best described with main sections for each study. Headings should be restricted to center headings for main sections and run-in italicized headings for subsections.

Tables. Data should be condensed and arranged to facilitate the comparisons the reader must make. Tables should be kept reasonably small by breaking up large ones, omitting all unessential data, and minimizing number of significant figures. Leader tables without number, main heading, or ruled lines are useful for small groups of data. Textual matter in tables should be minimized and unnecessary footnotes should be avoided.

Tables should be typed on separate pages at the end of the manuscript and their positions should be indicated to the printer by typing "(Table I)" in the appropriate place between lines of the text. (Figures are treated similarly.)

Figures. If possible, all drawings should be made by a competent draughtsman. Curves should be drawn heaviest, axes or frame intermediate, and grid lines lightest. The horizontal axis should be used for the independent variable; and experimental points should be shown. Labels are preferable to legends. All drawings should be made two to three times eventual reduced size with India ink on white paper, tracing linen, or blue-lined graph paper. Lettering should be done with a guide, and letters should be $\frac{1}{16}$ to $\frac{1}{8}$ inch high after reduction.

For difficult photographs, a professional should be hired or aid obtained from a good amateur. The subject should be lighted to show details. A bright print with considerable contrast reproduces best, and all prints should be made on glossy paper.

Figures should be identified by lightly writing number, author, and title on the back. Cut-lines (captions) should be typed on a separate sheet attached to the end of the manuscript.

Style. Clarity and conciseness are the prime essentials of a good scientific style. A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*.

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EFFECT OF SURFACE-ACTIVE AGENTS ON THE SOFTNESS AND RATE OF STALING OF BREAD¹

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ABSTRACT

The relative effectiveness in bread of 24 different surface-active agents as emulsifiers or "softeners" was determined. The results of compressibility tests and organoleptic examinations made on bread showed that five of the surface-active agents were more effective than the others. They were glyceryl monostearate, glyceryl oleostearate, polyoxyethylene stearate, sorbitan monostearate, and polyoxyethylene sorbitan monostearate.

Additional compressibility and organoleptic data obtained from commercial-scale baking tests of the five more effective emulsifiers showed that polyoxyethylene stearate was the most effective bread softener of the emulsifiers investigated. It also brightened the crumb color and improved the grain and texture of bread. The four other selected agents had similar bread-improving properties, but it was necessary to use larger quantities. Bread containing polyoxyethylene stearate had a lower rate of increase in crumb firmness upon storage than the control (no emulsifier added) bread.

Bread containing polyoxyethylene stearate or glyceryl monostearate when stored at 10°C. was not as soft from 12 to 96 hours after baking as control bread stored at approximately 28°C. However, the difference in degree of softness between control bread and those containing an emulsifier was greater at 10°C. than at 28°C.

Control bread 48 hours old was refreshed by heating to 60°C. in a radio-frequency field. It was not as soft at any time from 12 to 72 hours after refreshing as unheated bread of the same age containing polyoxyethylene stearate, glyceryl monostearate, or glyceryl oleostearate. Refreshed loaves containing emulsifiers had a greater rate of increase in crumb firmness upon storage than the respective unheated loaves. The rate was the same for refreshed and unheated control bread.

The addition of emulsifiers did not affect the rate of moisture loss in bread during a 96-hour staling period.

The homogenization at 1,000 and 3,000 p.s.i. of the sugar, nonfat dry milk solids, yeast food, and part of the water was ineffective as a method of making soft bread having improved crumb color, texture, and grain. Homogenizing glyceryl monostearate with these ingredients did not improve its effect.

¹ Manuscript received October 25, 1948.

² The Great Atlantic and Pacific Tea Company, National Bakery Division, New York 17, N. Y.

During the past several years the baking industry has become increasingly conscious of surface-active agents. Claims have been made that various compounds having surface-active properties are effective adjuncts in producing soft bread and in delaying the staling of baked products. Several workers have evaluated certain of these compounds. Carlin, Hopper, and Thomas (2) reported that a relationship exists between increasing firmness of the crumb and staling phenomena; that increasing firmness in bread crumb is a linear function of storage time; and that modified fats affect the slope of the staling curve. Favor and Johnston (5) reported that polyoxyethylene stearate is a crumb-softener with the ability to decrease the rate at which bread crumb becomes hard with age. Freilich (6) found that polyoxyethylene stearate decreased the staling rate of bread crumb, as measured by a compressibility method (but not as measured by the farinograph). This paper reports an investigation of the effect on bread of 24 different surface-active agents in general and five in detail.

As reviewed by Cathcart (3) and Geddes and Bice (7) bread stales rapidly below 60°C. and above 0°C. Work was done to learn whether the addition of emulsifiers would nullify the firming effect a lower temperature (10°C.) has on bread crumb. Such information would have practical application in areas where bread is transported at low temperatures.

Bread that has staled may be refreshed by heating to 60°C. or above (3, 7). It was of interest to study the effect emulsifiers have on bread refreshed by heat in a radio-frequency field. Moisture loss rates of refreshed and unheated bread were determined.

It has been stated that high-pressure homogenization of certain of the ingredients will keep bread soft and delay staling. Because like claims have been made for emulsifiers and the process of homogenization, their effect on bread has been compared.

Materials and Methods

Emulsifiers Tested. Table I lists the 24 different surface-active compounds investigated with a rating of their relative effectiveness in bread. This paper refers to the surface-active agents reported as bread emulsifiers or "softeners," although many of these compounds had been previously listed as suitable for all surface-active needs (8). During the preliminary survey of these surface-active agents the possible toxicity of many of them was ignored. After the initial testing all but five of the compounds were eliminated because of their ineffectiveness or because they imparted a foreign flavor to bread. The five selected compounds, glyceryl monostearate, glyceryl oleostearate, polyoxyethylene stearate, sorbitan monostearate, and polyoxyethylene

sorbitan monostearate, were studied further. Although polyethylene glycol (mono) laurate was rated excellent in effectiveness it was eliminated because it is closely related chemically to polyoxyethylene stearate and is no more effective. Glyceryl monostearates containing different percentages of mono-compound, manufactured with and without the addition of soap, dry and emulsified with water, were tested. Polyoxyethylene stearate manufactured by two different

TABLE I
SURFACE-ACTIVE AGENTS TESTED IN BREAD

Surface-active agent	Effectiveness in bread	Comments
Polyoxyethylene stearate	Excellent	—
Polyethylene glycol (mono) laurate	Excellent	—
Glyceryl (mono) oleate	Good	—
Glyceryl monostearate	Very good	—
Lecithin	Good	—
2-amino-1-butanol	Good	—
2-amino-2-methyl-1-propanol	Good	Imparts foreign odor to bread
Tris (hydroxymethyl) aminomethane	Poor	Ineffective in bread
2-aminoethyl ethanolamine	Fair	Alkaline reaction in bread
Diethylethanolamine	Good	Imparts foreign odor to bread
Monoethanolamine	Poor	Ineffective in bread
Morpholine	Fair	—
Sodium salt of higher secondary alkyl sulfate	Fair	—
Triethylene tetramine	Poor	Ineffective in bread
Diethylenetriamine	Fair	Imparts foreign odor to bread
Diethanolamine	Fair	Imparts foreign odor to bread
Triethanolamine	Fair	—
Polyoxyethylene ether of partial lauric acid ester	Good	—
Polyoxyethylene ether of partial palmitic acid ester	Good	—
Polyoxyethylene ether of partial stearic acid ester	Good	Used in mixture X
Polyoxyethylene ether of partial oleic acid ester	Good	—
Sorbitan monostearate	Good	Used in mixture X
Propylene glycol monostearate	Good	—
Glyceryl oleo stearate	Very good	—
Mixture X (88% sorbitan monostearate, 12% polyoxyethylene sorbitan monostearate)	Very good	—

processes and several brands of glyceryl oleostearate were tested. A mixture "X," composed of 88% sorbitan monostearate and 12% polyoxyethylene sorbitan monostearate, was made from two of the compounds selected for study.

Polyoxyethylene stearate and glyceryl oleostearate are plastic at 28°C., resemble firm lard, and incorporate readily with the other bread ingredients. Polyoxyethylene stearate was used alone, in a flour dispersion (75% flour plus 25% active ingredients), and emulsified

with 33.3%, 50%, and 75% of water. Neither polyoxyethylene stearate nor glyceryl oleostearate increased the water-absorbing properties of the dough. Glyceryl monostearate in flake and bead form was tested; it was employed dry and in water emulsions containing 25% and 33.3% emulsifier. Glyceryl monostearate increased water absorption when it was added to a dough. Mixture "X" was dispersed in flour (75% flour plus 25% active ingredients). The active ingredients of mixture "X" did not change dough absorption.

Baking Procedures. In both laboratory and commercial baking tests the sponge and dough process was used. The flour used throughout was hard winter wheat patent. The emulsifiers, the lard, and 60% of the total flour were included in the sponge. In all series a control made without an emulsifier was included. The laboratory baking test loaves weighed 16 oz. and yielded 18 slices approximately 0.4 inch thick. Commercial test loaves weighed 24 oz. and gave 25 slices approximately 0.5 inch thick. All loaves were sliced in a commercial type machine, wrapped, and weighed 90 minutes after baking. Laboratory-baked bread was wrapped in 300 MSAT³ cellophane and commercial bread in opaque waxed paper. Except for the preliminary screening of the emulsifiers and the homogenization study, tests were made on bread produced from commercial size (1,000 lb.) doughs.

The selected emulsifiers were added to dough in quantities of 0.25%, 0.5%, 0.75%, 1.0%, 1.5%, and 2.0% (based on weight of flour as 100%). This was approximately equivalent to 0.156%, 0.312%, 0.468%, 0.624%, 0.936% and 1.25% of emulsifier in the baked bread. All were tested to find whether their effectiveness differed when added to the sponge or to the dough stage.

Organoleptic Tests. During the earliest part of the work the softness of bread was determined by squeezing the loaf by hand, feeling the texture of the crumb, eating the bread, and mashing the cut surface of the loaf with the fist and noting its resiliency. The organoleptic examinations were made by a panel usually consisting of five laboratory workers, all of whom were familiar with bread-quality factors. Test bread was compared with a control of the same age. Preferences were not scored numerically, but relative ratings were noted.

Compressibility Procedure. In the later studies the Bloom Gelometer, having a modified plunger of 1 inch diameter, was used, in addition to the organoleptic tests, to determine the compressibility of bread crumb (2). The numerical values obtained with this instrument made possible the plotting of graphs illustrating the degree and rate of change in crumb firmness. The Bloom Gelometer measures

³ MS—Moistureproof; S—Heat Sealing; A—Anchored (Water Resistant); T—Transparent (Uncolored).

the weight in grams required to cause a circular plunger, 1 inch in diameter, to depress the surface of a 1 inch (25.4 mm.) thickness (i.e., two slices 0.5 inch thick) of bread by 4 mm. The firmer the crumb the greater the weight required to depress the surface 4 mm. Compressibility tests were also made with the A.S.T.M. Penetrometer, but it was found that the Bloom Gelometer has less shearing effect on the crumb than the penetrometer.

When making compressibility tests the end slices of the loaf were not used. The crumb surface of every second slice was tested and two slices were placed together for testing on the instrument. The plunger was in contact with the crumb at a distance approximately 1 inch from the bottom and side crust of the slice. The compressibility value obtained was the average of eight different compressibility tests for the 16-oz. and 10 for the 24-oz. loaf. In most series compressibility tests were made 12, 24, 48, 72, and 96 hours after baking. In many the first test was made 90 minutes after baking, then at 12 hours, and every 12-hour period thereafter until 96 hours had elapsed. In some series the time was extended to 120 hours.

Storage at Different Temperatures. The breads containing polyoxyethylene stearate and glyceryl monostearate and a control were stored, 90 minutes after baking, at room temperature (approximately 28°C.) and at 10°C. Compressimetric and organoleptic tests were made during a 96-hour period. The bread stored at 10°C. was tested immediately upon removal from storage and also after being warmed to room temperature. (Six hours were required for the temperature to rise from 10°C. to 28°C.)

Refreshing of Bread. The breads containing polyoxyethylene stearate, glyceryl monostearate, and glyceryl oleostearate and a control were stored at room temperature (approximately 28°C.) for 48 hours and then refreshed by heating in a radio-frequency field. The interior of the bread was heated to 60°C. in a 25 megacycle field. This method was used so that the bread could be kept wrapped during heating, thereby preventing undue moisture loss. The same general method as was used by Cathcart, Parker, and Beattie (4) was employed. Compressibility and organoleptic tests were made after the bread had cooled to 30°C. (6 hours after heating); and at 72, 96, and 120 hours after baking.

Moisture Retention. The moisture content of bread was determined to learn the effect of the emulsifiers on the moisture retention of bread. Ninety minutes after baking and immediately after slicing and wrapping, the bread was weighed to 0.1 g. The loaves tagged for the determination of moisture were reweighed before each compressibility test period. After the final test period the moisture content of the bread

was determined (1). The moisture content of the bread at the time of each compressibility test was determined by using as an initial weight that obtained at the time of the compressibility test.

Homogenization. The sugar, nonfat dry milk solids, yeast food, and part of the water were homogenized at 1,000 and 3,000 p.s.i. in a Manton-Gaulin two-stage homogenizer. Other emulsions were made containing glyceryl monostearate in addition to these ingredients.

Results and Discussion

Effectiveness of Emulsifiers. The average results of organoleptic tests and the compressibility values obtained on more than 100 different laboratory and commercial baking tests showed that polyoxyethylene stearate kept bread softer for a longer period of time than bread made without it. It also brightened the crumb color slightly and improved the grain and texture of bread. With the bread formula used, 0.5% polyoxyethylene stearate was sufficient to give the maximum softening and improving effect. The same quantities of glyceryl monostearate and glyceryl oleostearate were not as effective as polyoxyethylene stearate. However, both were effective in keeping bread softer than

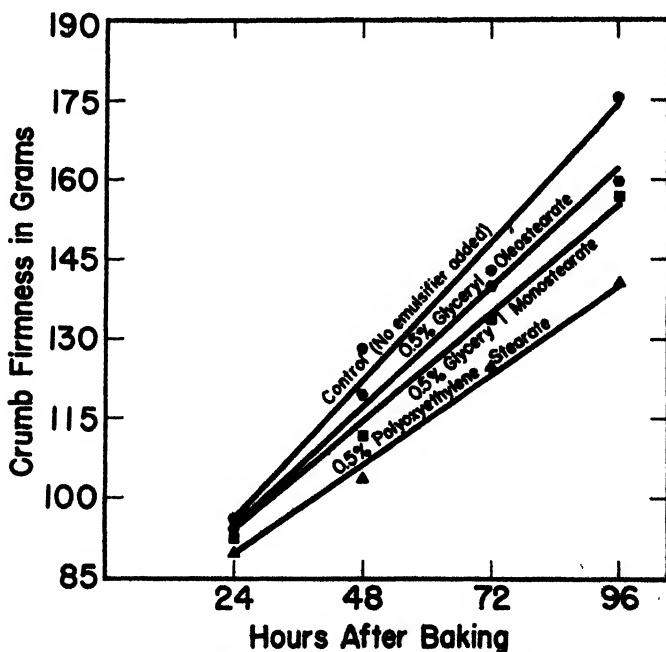


Fig. 1. Effect of 0.5% (flour basis) of various emulsifiers on the degree and rate of increase in crumb firmness using the Bloom Gelometer. Glyceryl oleostearate and polyoxyethylene stearate, both in plastic form (100% active), and 1.5% of a water emulsion of glyceryl monostearate (33.3% active) were used.

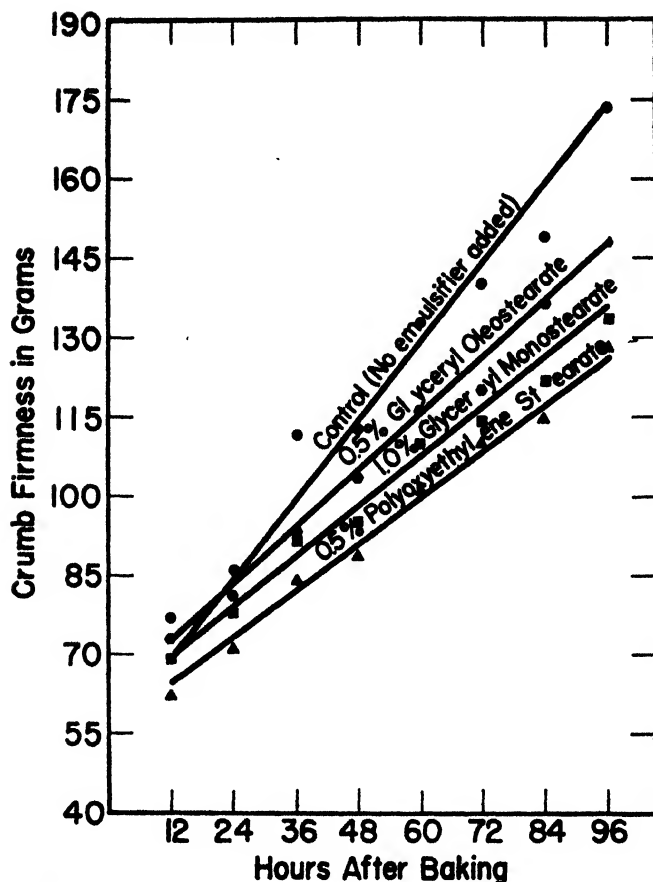


Fig. 2. Effect of emulsifiers on the degree and rate of increase in crumb firmness using the Bloom Gelometer. Glyceryl oleostearate and polyoxyethylene stearate, both in plastic form (100% active), and 3.0% of a water emulsion of glyceryl monostearate (33.3% active) were used.

the control. Bread containing 1.0% glyceryl monostearate approached but did not equal the softness of crumb of bread containing 0.5% polyoxyethylene stearate (Figs. 2 and 3). Mixture "X" was not as effective as the same quantities of polyoxyethylene stearate, glyceryl monostearate, or glyceryl oleostearate but made bread softer than the control. Polyoxyethylene stearate was equally effective whether used in a plastic condition or dispersed in flour or water. Glyceryl monostearate showed almost the same bread softening properties when added dry to the dough that it did when premixed (emulsified) with water. Glyceryl monostearate was most effective when twice its weight in extra water was added to the dough. The same relative rating as emulsifiers was obtained for polyoxyethylene stearate.

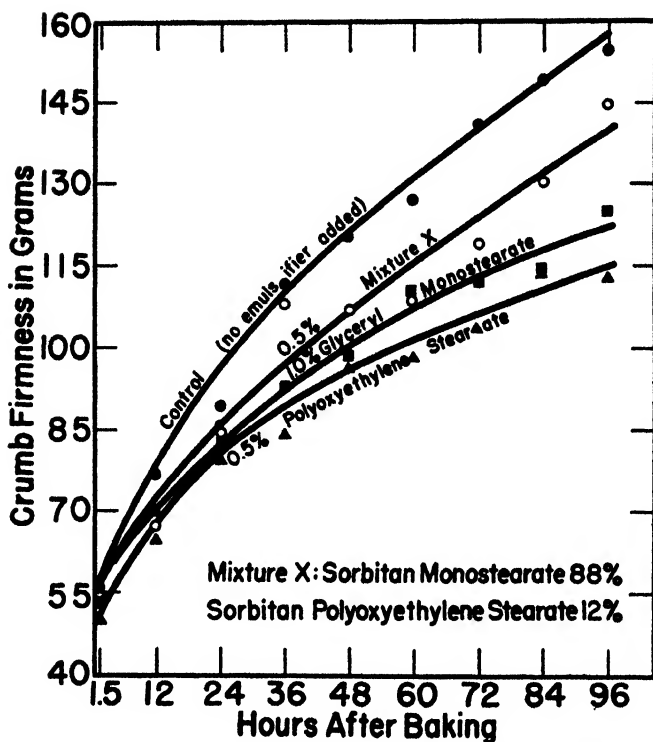


Fig. 3. Effect of emulsifiers on the degree and rate of increase in crumb firmness using the Bloom Gelometer. Polyoxyethylene stearate in plastic form (100% active), 3.0% of a water emulsion of glyceryl monostearate (33.3% active), and 2.0% of a flour dispersion of mixture X (25% active) were used.

rate, glyceryl monostearate, glyceryl oleostearate, and mixture "X" whether they were added to the sponge or to the dough stage. Polyoxyethylene stearates manufactured by two different processes were equally effective as bread softeners and improvers.

Favor and Johnston (5) reported that polyoxyethylene stearate, in quantities up to 2.0% of the flour weight, had no significant effect on bread doughs during processing or on the physical character of the bread other than its softness and tenderness. It was noted during the work reported here that the addition of 0.5% of this material to dough improved its machining properties. As mentioned previously, it also brightened the crumb color and improved the grain and texture of bread. When processed and baked under comparable conditions, bread containing polyoxyethylene stearate had a slightly darker crust color than the control. The emulsifiers had little effect on bread volume, and in any particular series, the loaf volumes were the same, within experimental error.

Figs. 1, 2, and 3 illustrate the effect of polyoxyethylene stearate, glyceryl monostearate, glyceryl oleostearate, and mixture "X" on bread crumb firmness (compressibility) during 96-hour staling periods. These are typical curves obtained on particular days and are representative of the results obtained in many series. Fig. 3 shows that 90 minutes after baking, differences in compressibility value are slight; and at the beginning the curves are not straight lines. The difference in the firmness (compressibility) between bread containing an emulsifier and the control is very small within a few hours after baking but becomes significant by 48 hours. Differences in the degree of softness were first observable, by organoleptic methods, 12 hours after baking.

Some workers have placed emphasis on the slope of the curves obtained from compressibility data, believing that the slope differentiates between the effect of various emulsifiers on the rate at which bread crumb becomes firm. Only after repeated tests may such conclusions be drawn, because the results of baking tests are affected by many complex factors. Differences in fermentation time, temperature, and humidity, in ingredients, in scaling weight, and in baking and cooling conditions will affect compressibility data. As a result of the many tests conducted, typical examples of which are reported here, it was found that bread containing 0.5% polyoxyethylene stearate had a lower rate of increase in crumb firmness upon storage than control bread. The slope of the curves obtained from bread containing 0.5% of glyceryl monostearate, glyceryl oleostearate, and mixture "X" indicated a rate of increase in crumb firmness which was intermediate between the control bread and that containing polyoxyethylene stearate. The differences in rate for equal quantities of glyceryl monostearate, glyceryl oleostearate, and mixture "X" are not believed significant; i.e., the differences were not easily observed when checked by organoleptic tests. However, all the emulsifiers selected for study kept bread softer than the control.

Effect of Different Storage Temperatures. Fig. 4 illustrates the effect of storage at 10°C. and at room temperature (approximately 28°C.) on the degree and rate of increase in crumb firmness during a 96-hour staling period. From 12 hours to 96 hours after baking all bread stored at 10°C. and warmed to room temperature before compressibility tests were made had a firmer crumb than the respective bread stored continuously at room temperature. In all cases the firmness of crumb was exaggerated when bread stored at 10°C. was tested immediately upon removal from storage. From these results it is concluded that the increased firmness effect of low temperatures (10°C.) on bread crumb cannot be compensated for by the addition of

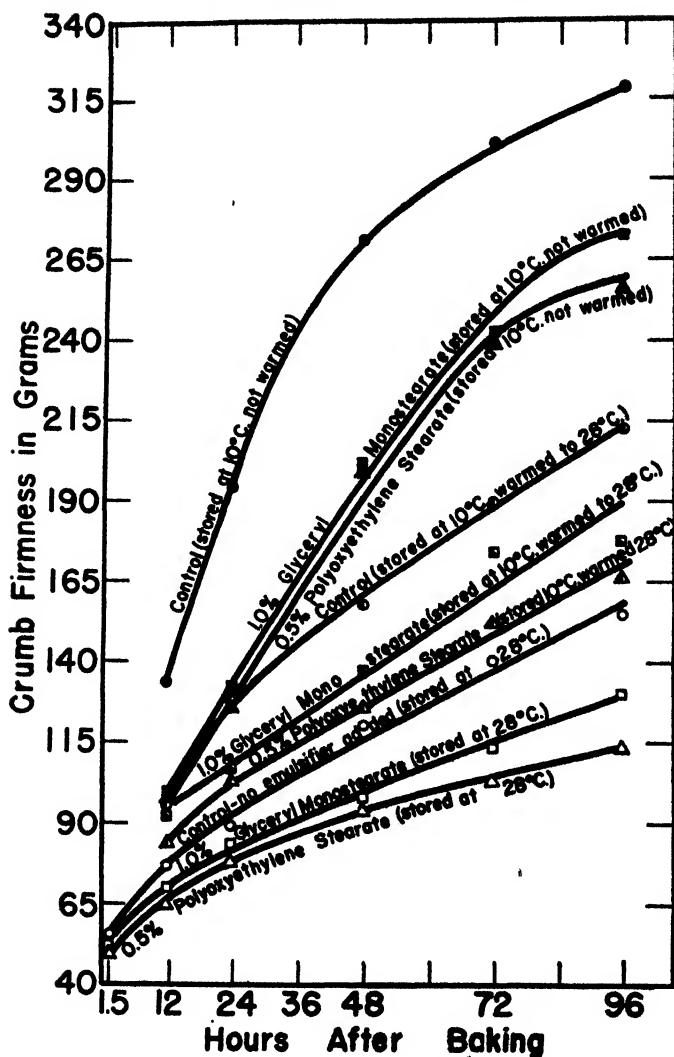


Fig. 4. Effect of emulsifiers on the degree and rate of increase in crumb firmness of bread stored at 10°C. and at room temperature (approximately 28°C.) using the Bloom Gelometer. Polyoxyethylene stearate in plastic form (100% active) and 3.0% of a water emulsion of glyceryl monostearate (33.3% active) were used.

emulsifiers. However, bread containing an emulsifier will maintain the advantage of having a softer crumb than the control when both are stored at 10°C.

Effect of Refreshening Bread. When breads containing polyoxyethylene stearate, glyceryl monostearate, and glyceryl oleostearate and a control were heated to 60°C. in a radio-frequency field, 48 hours after baking, all were freshened to the extent that they appeared to

be oven-fresh. Compressibility tests made after the bread had cooled in 6 hours to 30°C. showed that the treatment had a softening effect on all samples (Fig. 5). Twelve hours after heating (60 hours after baking) all refreshed loaves were softer than the respective unheated loaves. However, by 48 hours after heating (96 hours after baking)

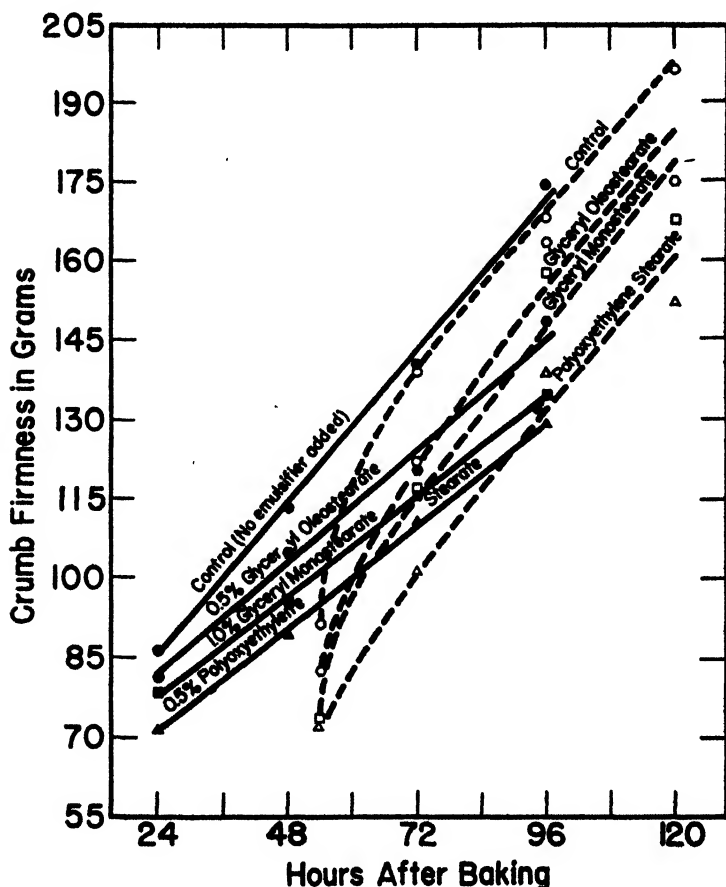


Fig. 5. Effect of emulsifiers on the degree and rate of increase in crumb firmness of bread heated to 60°C. in a radio-frequency field. Solid lines represent unheated bread; broken lines represent refreshed (heated) bread. Glycerol oleostearate and polyoxyethylene stearate, both in plastic form (100% active), and 3.0% of a water emulsion of glycerol monostearate (33.3% active) were used.

all the electronically heated loaves containing emulsifiers had lost the advantage of being softer than the respective unheated loaves. From 60 hours after baking onward, unheated breads containing emulsifiers were softer than the refreshed control.

Bread wrapped in 300 MSAT cellophane lost moisture when heated to an internal temperature of 60°C. in a radio-frequency field. The

effect of refreshing on the moisture content of bread containing polyoxyethylene stearate and glyceryl monostearate and a control is shown in Fig. 6. The rate of moisture loss for all bread was increased after heating; however, the relative position of the curves remained the same.

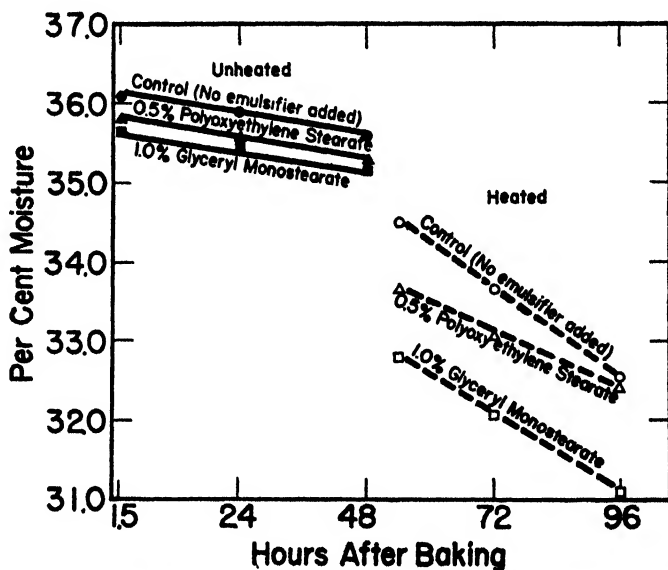


Fig. 6. Effect of emulsifiers on the rate of moisture loss of bread before and after refreshing at 60°C. in a radio-frequency field. Polyoxyethylene stearate in plastic form (100% active) and 3.0% of a water emulsion of glyceryl monostearate (33.3% active) were used.

Effect of Emulsifiers on Moisture Retention. Moisture determinations were made on many of the bread series and showed that, with normal baking temperature and time, the addition of polyoxyethylene stearate or glyceryl monostearate did not affect the rate of moisture loss. A typical example is shown in Fig. 7. The rate of moisture loss was the same, within experimental error, for bread containing emulsifiers and the control. In some series, bread containing glyceryl monostearate had a slightly higher moisture content than either that made with polyoxyethylene stearate or the control.

Effect of Homogenization on Softness. The homogenization of various combinations of bread ingredients at 1,000 and 3,000 p.s.i. did not make bread as soft as that containing any of the five selected emulsifiers. Compressibility data showed that bread having certain ingredients homogenized had as firm a crumb as control bread during a 96-hour staling period. The homogenization of glyceryl monostearate with the ingredients usually homogenized did not improve its effectiveness in bread.

Meaning of Softness. During the staling of bread the crust becomes soft and leathery; the crumb becomes firm, harsh, and crumbly to the touch; and the bread loses moisture. Many tests have been developed and described in the literature for measuring one or more of these changes. From previous work done by the authors it is believed that compressibility testing resembling that described approaches more closely the consumer's concept of freshness than any other test. In our opinion the consumer's criterion is softness of crumb and, in general, softness and good mastication properties go together. Good

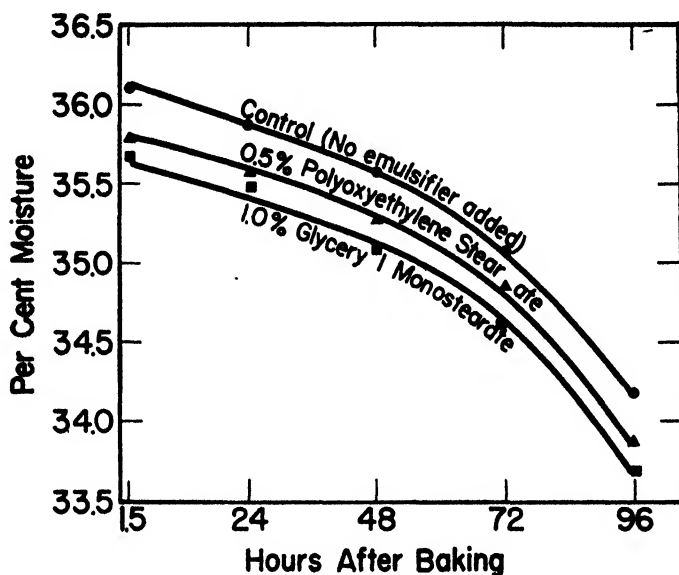


Fig. 7. Effect of emulsifiers on the rate of moisture loss of bread. Polyoxyethylene stearate in plastic form (100% active) and 3.0% of a water emulsion of glyceryl monostearate (33.3% active) were used.

correlation was noted between compressibility data obtained with the Bloom Gelometer and the "squeeze" test. Gelometer values when shown graphically reveal differences in softness that correlate with the consumer's "squeeze test." Curves showing penetrometer values do not illustrate such differences to the same extent. Although the results of this compressibility procedure do not correlate perfectly with mastication properties, the method is one of the best available at present. Crumbliness determinations were not made, but organoleptic tests showed that the five emulsifiers which were studied in detail tended to make bread more crumbly at the start. At no time, however, did this factor become undesirable, and it was felt that it was more a crumbliness that is associated with a rich formula than that which is associated with staleness.

Acknowledgments

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DIFFERENTIAL STABILITY OF ALPHA-AMYLASE AND PROTEINASE ¹

BYRON S. MILLER and JOHN A. JOHNSON

ABSTRACT

Investigations of the differential stability of alpha-amylase and proteinase employing malted wheat flour, malted barley, and commercial fungal concentrates showed that the hydrogen-ion activity of the extracts was the most important factor influencing the stability of both enzymes.

Alpha-amylase was stable on standing for 20 hours at 5°C. over a rather narrow range of pH, depending on the enzyme source. Proteinase was stable over a wide pH range under these same conditions. The differential effect of pH on enzyme stability (5°C., 20 hours) was utilized as a means of separating alpha-amylase and proteinase in malted wheat flour and malted barley. This technic was not applicable to the fungal preparation.

Differential thermal inactivation studies at various hydrogen-ion concentrations (pH 3.0-10.5) demonstrated that the stability of both enzyme systems was markedly affected by relatively high temperatures, 50° to 60°C.

Optimum conditions for the differential inactivation of proteinase by heating at 50°C. for 30 minutes were a pH of 9.3 for malted barley, 10.2 for malted wheat flour, and 10.5 for the fungal preparation. Under these conditions complete inactivation of the proteinase could be obtained with a maximum retention of 80% of the alpha-amylase. Similar treatment employing a pH of 3.6 resulted in complete inactivation of the alpha-amylase and a maximum retention of 66% of the proteinase. Conditions for the differential inactivation of alpha-amylase and proteinase in fungal preparations were variable depending on the preparation tested.

Within wide limits, the enzyme stability did not appear to be a function of enzyme concentration. The concentration of accompanying substances, however, appeared to be a factor of prime importance bearing on stability.

The evaluation of the significance of both alpha-amylase and proteinase in breadmaking and other fermentation industries has been complicated by the lack of suitable means for separating or differentially inactivating either of these two enzyme systems. Preparations of proteinase and alpha-amylase uncontaminated with each other would be useful agents for further research. Certain accepted concepts may

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be modified or perhaps new ones evolved if experiments were performed using such enzyme systems. The development of technics whereby such preparations could be obtained easily and practicably was the primary goal of this investigation.

Research by many workers summarized by Caldwell and Adams (1) and Kneen, Sandstedt, and Hollenbeck (5) shows that alpha-amylase from various sources is more sensitive to high hydrogen-ion activities than is beta-amylase. This property serves as a means for separating the alpha and beta components. In contrast, Mounfield (9) reported that wheat proteinase is relatively stable in buffer solutions maintained as low as pH 4, but is destroyed at pH 8 at 18°C. in less than 3 days.

In an extensive study of the separation of the alpha- and beta-amylase components of malt, Kneen, Sandstedt, and Hollenbeck (5) defined the conditions of time, temperature, pH, and the presence or absence of supplementary calcium-ions necessary for the differential inactivation of either component. Maximum retention of alpha-amylase (90 to 100%) and complete inactivation of beta-amylase were attained in the presence of calcium-ion at a pH of 6.0 to 7.0 and a temperature of 70°C. Conversely, maximum retention of beta-amylase and complete inactivation of alpha-amylase were attained in the absence of calcium-ion at a pH of 3.0 and a temperature of 30°C. The influence of these conditions on the activity of proteinase was not investigated.

Hildebrand (3), Miller and Johnson (7), and Dirks and Miller (2) have reviewed the literature dealing with means of separating the proteinase from amylolytic preparations. Most early workers have relied on the specific action of safranine to remove the proteinases from amylolytic preparations. Dirks and Miller (2) investigated a number of adsorbents with respect to their ability to remove proteolytic enzymes selectively. Associated treatments including the influence of high hydroxyl-ion concentration were also investigated. From 50 to 60% of the proteolytic activity in a mold bran extract was inactivated irreversibly by the adjustment of the hydrogen-ion concentration to pH 10.0. Under these conditions little decrease in amylolytic activity occurred. Similar effects were noted for extracts of malted wheat flour and malted barley.

The present investigation was undertaken following the promising results obtained in the previous study by Dirks and Miller (2) using high hydroxyl-ion concentrations to remove the proteinase from fungal extracts, and the work of Kneen, Sandstedt, and Hollenbeck (5) concerning the stability of amylases. Included in the study were the effects of time, temperature, and hydrogen-ion concentration on the

differential stability of alpha-amylase and proteinase in various amylolytic preparations.

Materials and Methods

Three enzyme sources were investigated. These included a commercial malted wheat flour, a five-day malted barley, and a commercial fungal concentrate possessing 42.8, 81.4, and 3300 alpha-amylase units per gram, respectively. In addition, a series of malted wheat flours, malted barleys, and several fungal preparations having a wide range of enzyme activity was studied.

Enzyme solutions were prepared by a one-hour extraction (30°C.) of the original preparation. Adjustment to a pH value appropriate for maximum stability was effected as soon as practicable. The fungal concentrate was extracted in the ratio of 2 mg. per ml. of extractant. The malted wheat flour and the malted barley (freshly ground on a Wiley mill using a 1 mm. screen) were extracted in the ratio of 2 g. to 5 ml. of liquid. Solutions from which the proteinase was to be removed were extracted and diluted with 0.2% calcium chloride solution. Solutions from which the alpha-amylase was to be removed were extracted and diluted with distilled water.

All pH values were measured or adjusted by the use of glass electrode equipment. Instrument calibrations were performed using a saturated solution of potassium acid tartrate (pH 3.7) and Clark and Lubs buffer solutions. Readings over the entire range were checked against readings obtained with a second instrument.

The effects of temperatures ranging from 5° to 60°C. and of hydrogen-ion concentrations from pH 3.0 to 10.5 were investigated. In all cases care was exercised to insure uniformity of treatment and minimal loss by evaporation. The pH values of the solutions were changed arbitrarily by the addition (with rapid mechanical stirring) of either 1.0 *N* sodium hydroxide or 1.0 *N* sulfuric acid. All solutions were re-adjusted to 30°C. and to a stable pH after treatment and before enzyme activities were determined.

Alpha-amylase determinations were made according to the procedure of Sandstedt, Kneen, and Blish (10). Determinations of proteolytic activity were made using the Ayre-Anderson procedure as modified by Miller (6).²

Results and Discussion

Stability of Alpha-Amylase and Proteinase at Various pH Levels. The apparent greater proteinase activity for preparations extracted

² It is recognized that the method for determining proteolytic activity using hemoglobin as an auxiliary substrate is empirical and may or may not be correlated with proteolysis of other substrates.

with 0.2% calcium chloride (pH 6.0) compared with extractions made with distilled water (pH 7.6) and the previous work reported by Mounfield (8) led to a study of the stability of the enzymes in solutions of various hydrogen-ion concentrations. It has been established earlier by Dirks and Miller (2) that 0.2% calcium chloride did not influence the proteinase activity appreciably. A graphical summary of the data obtained at various pH values with extracts of malted wheat

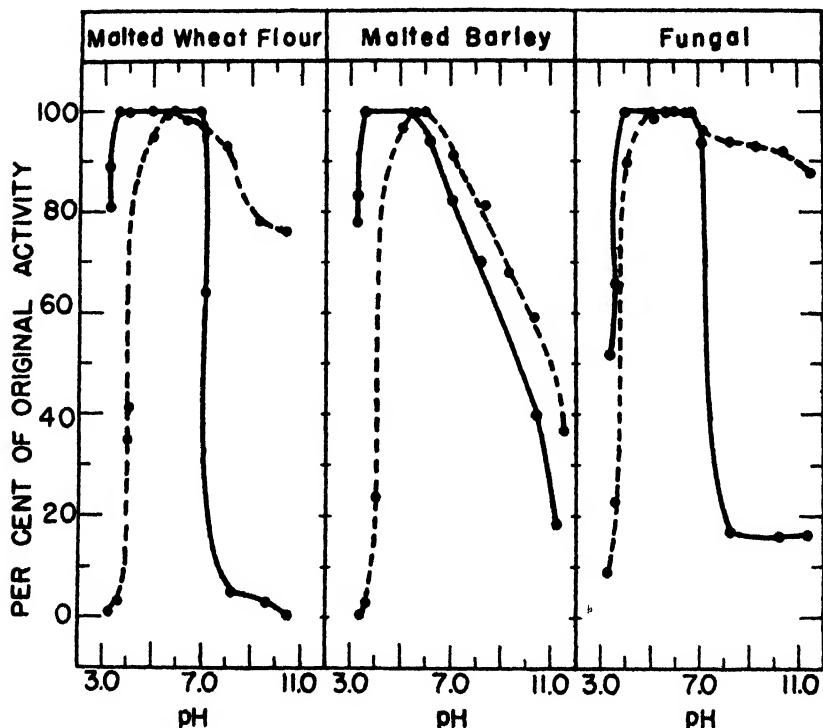


Fig. 1. Stability of alpha-amylase and proteinase in extracts of malted wheat flour, malted barley, and a fungal preparation standing at various hydrogen-ion concentrations (20 hours, 5°C). Solid lines represent retention of proteinase and dashed lines represent retention of alpha-amylase.

flour, malted barley, and a fungal concentrate is presented in Fig. 1. All solutions stood 20 hours in a refrigerator (5°C.) at the indicated pH.

Mounfield (8) found that the storage of aqueous extracts of sprouted wheat in the dark at 18°C., in the presence of toluene, resulted in a progressive loss of proteolytic activity such that one-half of the original activity was lost in 18 days. This aging process was accompanied by a deepening of the extract color, the appearance of a precipitate, and a decrease in pH value. The inactivation was found to follow the course of a unimolecular reaction. The results obtained

in the present study indicate that this inactivation can be speeded up by the selection of an appropriate pH.

The relative positions of the activity curves for malted wheat flour and malted barley (Fig. 1) show that this technic may be applied as a means of differentially inactivating the alpha-amylase in both malted wheat flour and malted barley. This method, however, does not permit the differential inactivation of the proteinase in malted barley, although it appears to be effective for malted wheat flour.

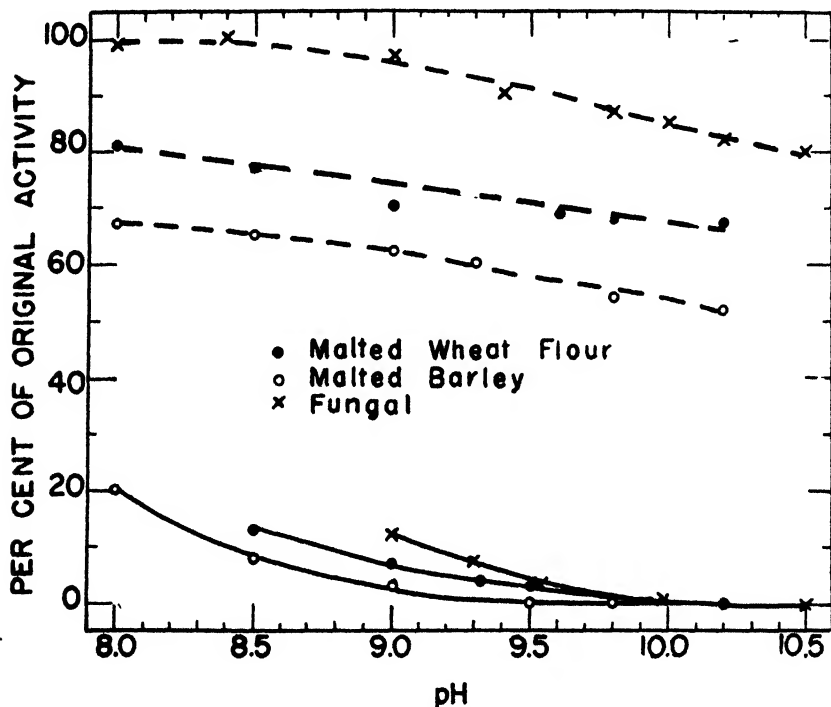


Fig. 2. Influence of high pH on the thermal inactivation (30 minutes, 50°C.) of proteinase in extracts containing alpha-amylase. Solid lines represent retention of proteinase and dashed lines represent retention of alpha-amylase.

The close proximity of the curves representing the activities of the two enzyme systems in the fungal preparation precludes the application of this means for differentially separating either alpha-amylase or proteinase in this particular sample. Preparations produced from the growth of other organisms or under different cultural practices may show different properties.

Differential Inactivation of Proteinase and Alpha-Amylase at High pH. A study of the manner in which alpha-amylase and proteinase are influenced by hydrogen-ion concentration necessarily has involved

a consideration of the time of contact under specified conditions of temperature and hydrogen-ion concentration. The percentages of alpha-amylase and proteinase remaining after heating extracts of malted wheat flour, malted barley, and a fungal amylase preparation at various low hydrogen-ion concentrations are shown in Fig. 2. These studies were conducted under conditions of high pH (8.0 or above) and involved heat treatment at 50°C. for 30 minutes such that the major

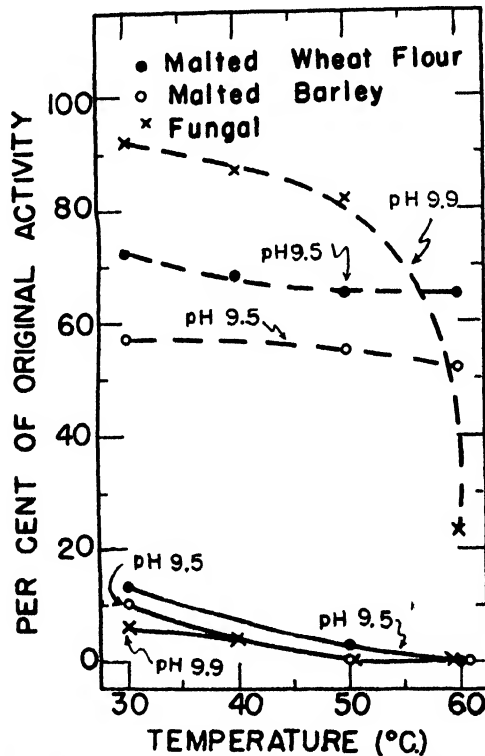


Fig. 3. Influence of temperature on the differential thermal inactivation of proteinase in extracts containing alpha-amylase. Solid lines represent retention of proteinase and dashed lines represent retention of alpha-amylase.

influence was on the proteinase system rather than on the alpha-amylase system.

It is apparent from these data that there is little difference in the susceptibility of the proteinase from the three preparations to inactivation at high pH. The most stable proteinase system was that present in the fungal preparation which required the adjustment of the pH to 10.5 in order to destroy the proteinase completely.

Fungal alpha-amylase also was more stable than that present in the other preparations. This is surprising in view of the known thermo-

lability of fungal amylase in the pH range of 5.0 to 6.0 (4). At pH values suitable for minimum retention of proteinase, approximately 80% of the fungal alpha-amylase was retained as compared with 60 to 65% in the malted wheat flour and malted barley, respectively.

The influence of temperature on the differential inactivation of proteinase at selected high pH values is graphically illustrated in Fig. 3. Heating at temperatures ranging from 50° to 60°C. caused little change in the amount of alpha-amylase retained in malted wheat flour

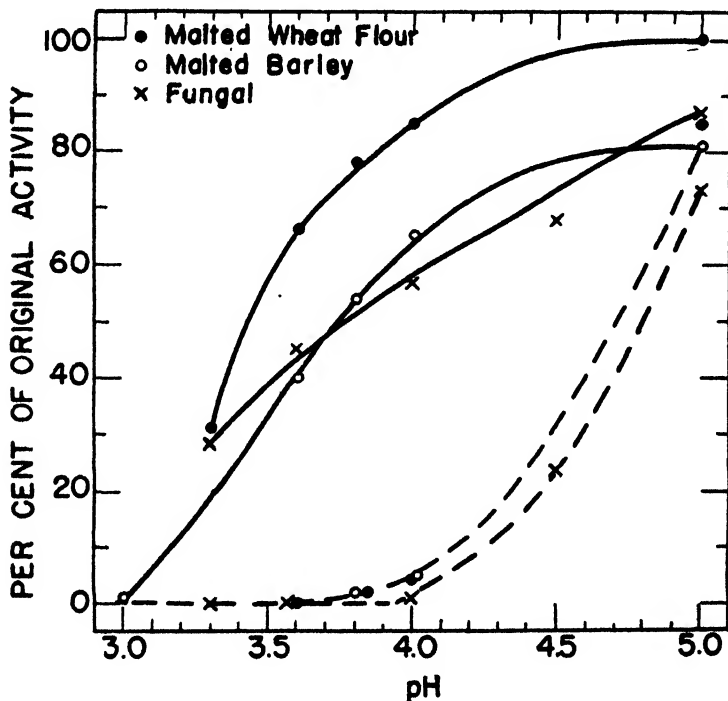


Fig. 4. Influence of low pH on the thermal inactivation (30 minutes, 50°C.) of alpha-amylase in extracts containing proteinase. Solid lines represent retention of proteinase and the dashed lines represent retention of alpha-amylase.

or malted barley extracts, but a sharp decrease in the amount of alpha-amylase retained by the fungal preparation. It was necessary to heat malted wheat flour extract at 60°C. to inactivate the last traces of proteinase. Inactivation of the proteinase in extracts of malted barley and of the fungal preparations, however, appeared to be essentially complete at 50°C.

The time of heating was not critical, although prolonged heating up to 60 minutes caused a slight decrease in the activity of both enzyme systems. An increase in heating time from 10 to 60 minutes

(50°C. and pH 9.5) resulted in a decrease in amylolytic and proteolytic activity for malted wheat flour amounting to 7 and 3%, respectively. For the fungal preparation adjusted to pH 9.9 and heated at 50°C. this decrease amounted to 5 and 0.5%.

Considerable flocculation of enzymatically inactive material occurred during the heating of the extracts at high pH (8.0–10.5). This technic might serve, therefore, as a preliminary purification step

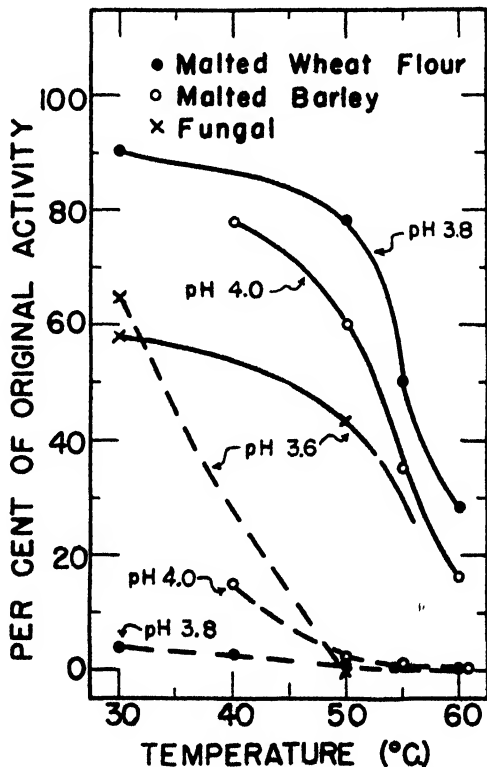


Fig. 5. Influence of temperature on the differential thermal inactivation of alpha-amylase in extracts containing proteinase. Solid lines represent retention of proteinase and dashed lines represent retention of alpha-amylase.

in the preparation of an amylase concentrate. Similarly, the heat treatment of extracts at low pH resulted in the flocculation of smaller but significant amounts of inactive material.

Differential Inactivation of Alpha-Amylase and Proteinase at Low pH. In crude aqueous extracts the alpha-amylase of malted wheat flour, malted barley, and fungal preparations was more sensitive to high hydrogen-ion activities than was the proteinase. This fact has been utilized in removing alpha-amylase from extracts containing

proteinase. Again, the manner in which this differential inactivation takes place necessarily involved a consideration of the time of contact under specified conditions of temperature and pH. The percentages of alpha-amylase and proteinase remaining in the three enzyme sources after heating the extracts (30 minutes, 50°C.) at various hydrogen-ion concentrations are shown in Fig. 4.

Heating (50°C. for 30 minutes) at a pH value of 3.6 was necessary to remove essentially all of the alpha-amylase from these preparations. Under these conditions 66% of the malted wheat flour proteinase, 39% of the malted barley proteinase, and 45% of the fungal proteinase were retained. Thus, malted wheat flour proteinase appears to be more stable to inactivation at low pH than that present in malted barley or in the fungal preparation. Heating at 50°C. for 30 minutes (pH 4.0) resulted in a substantial increase of proteinase retained by each prep-

TABLE I
OPTIMUM CONDITIONS FOR DIFFERENTIAL INACTIVATION OF
ALPHA-AMYLASE IN EXTRACTS CONTAINING PROTEINASE
(Percentages of original activity remaining)

Enzyme source	Treatment			Alpha-amylase	Proteinase
	[H ⁺]	Temp.	Period		
	pH	°C.	hrs.	%	%
Malted wheat flour	3.6	50	0.5	0.3	66
	3.6	5	20	3.5	80
Malted barley	3.3	50	0.5	0	29
	3.8	50	0.5	2	54
Fungal concentrate	3.6	5	20	0.4	85
	3.6	50	0.5	0	45

aration. There was, however, a concomitant small increase in the amount of alpha-amylase retained, varying from 1% for the fungal preparation to 4% for both the malted barley and malted wheat flour.

The effect of heating extracts at selected pH values for 30 minutes at different temperatures is illustrated in Fig. 5. The most significant result is the sharp decrease in the percentage of proteinase retained in the extracts when these preparations were heated at temperatures varying from 50° to 60°C.

Optimum Conditions for Inactivation of Proteinase or Alpha-Amylase. The optimum conditions for maximum retention of proteinase together with complete or nearly complete inactivation of alpha-amylase are summarized in Table I. It is possible, for each enzyme preparation, to obtain proteinase completely free from alpha-amylase, although there also may be considerable loss in proteinase activity.

In some cases, a better separation of alpha-amylase and proteinase was obtained by allowing the extract to stand in the refrigerator for 20 hours after adjustment to the proper pH value. In other instances, a heat treatment after adjustment to a selected pH value may be more desirable. The method employed depends on the degree of separation desired.

A summary of the data obtained for a series of malted wheat flours and malted barleys possessing a wide range of enzyme activity is presented in Table II. It would appear from these results that the

TABLE II

INACTIVATION OF ALPHA-AMYLASE FROM A SERIES OF MALTED WHEAT FLOURS AND MALTED BARLEYS POSSESSING A WIDE RANGE OF ENZYME ACTIVITY
(Percentages of original activity remaining)

Sample no.	Alpha-amylase	Proteinase activity	Treatment			Amylase	Proteinase
			[H ⁺]	Temp.	Time		
	<i>units</i>	<i>ml.</i>	<i>pH</i>	<i>°C.</i>	<i>hrs.</i>	<i>%</i>	<i>%</i>
MALTED WHEAT FLOURS							
A	30.8	1.61	3.6	50	0.5	0.3	65
B	60.0	2.12	3.6	50	0.5	0.3	77
C	85.7	2.52	3.6	50	0.5	0.6	79
D	109.1	2.83	3.6	50	0.5	0.5	66
A	30.8	1.61	3.6	5	20	3.6	84
B	60.0	2.12	3.6	5	20	3.4	81
C	85.7	2.52	3.6	5	20	3.7	78
D	109.1	2.83	3.6	5	20'	3.7	82
MALTED BARLEYS							
1	41.0	1.73	3.6	5	20	0.5	86
2	85.7	2.70	3.6	5	20	0.3	79
3	126.3	3.20	3.6	5	20	0.4	80
4	145.5	3.31	3.6	5	20	0.2	86
5	150.0	3.69	3.6	5	20	0.4	81

degree of differential separation of the enzymes bears no relation to the enzyme activities of the original preparations. Thus, heating an extract of malted wheat flour (pH 3.6, 50°C., 30 minutes) resulted in a loss of all but 0.3 to 0.6% of the alpha-amylase and a retention of 65 to 79% of the proteinase. Employing other conditions (pH 3.6, 5°C., 20 hours), similar uniformity was obtained but greater percentages of both enzymes were retained. Treating extracts of malted barleys similarly resulted in a loss of all but 0.2 to 0.5% of the alpha-

TABLE III
OPTIMUM CONDITIONS FOR DIFFERENTIAL INACTIVATION OF
PROTEINASE IN EXTRACTS CONTAINING ALPHA-AMYLASE
(Percentages of original activity remaining)

Enzyme source	Treatment			Alpha-amylase	Proteinase
	[H ⁺]	Temp.	Time		
	<i>pH</i>	<i>°C.</i>	<i>hrs.</i>	<i>%</i>	<i>%</i>
Malted wheat flour	9.5	60	0.5	65	0
	10.2	50	0.5	67	0
	10.0	5	20	65	0.1
Malted barley	9.3	50	0.5	60	0
Fungal concentrate	10.5	50	0.5	80	0

amylase and a retention of 79 to 86% of the proteinase. No conditions were found that were optimum for the differential separation of proteinase and alpha-amylase in several fungal preparations.

Maximum retention of alpha-amylase together with nearly complete inactivation of proteinase was best obtained under the optimum

TABLE IV
INACTIVATION OF PROTEINASE FROM A SERIES OF MALTED WHEAT FLOURS
AND MALTED BARLEYS POSSESSING A WIDE RANGE OF ENZYME ACTIVITY
(Percentages of original activity remaining)

Sample no.	Alpha-amylase	Proteinase activity	Treatment			Alpha-amylase remaining	Proteinase remaining
			[H ⁺]	Temp.	Time		
	<i>units</i>	<i>ml.</i>	<i>pH</i>	<i>°C.</i>	<i>hrs.</i>	<i>%</i>	<i>%</i>
MALTED WHEAT FLOURS							
A	30.8	1.61	9.5	60	0.5	67	0
B	60.0	2.12	9.5	60	0.5	74	0
C	85.7	2.52	9.5	60	0.5	61	0
D	109.1	2.83	9.5	60	0.5	60	0
A	30.8	1.61	10.0	5	20	64	0.1
B	60.0	2.12	10.0	5	20	67	0.1
C	85.7	2.52	10.0	5	20	64	0.1
D	109.1	2.83	10.0	5	20	61	0.1
MALTED BARLEYS							
1	41.0	1.73	9.3	50	0.5	66	0.1
2	85.7	2.70	9.3	50	0.5	60	0.1
3	126.0	3.20	9.3	50	0.5	56	0.1
4	145.5	3.31	9.3	50	0.5	62	0.1
5	150.0	3.69	9.3	50	0.5	60	0.1

conditions of pH, time, and temperature recorded in Table III. A summary of the percentages for both enzymes remaining after treatment of the series of malted wheat flours and malted barleys at these optimum conditions is shown in Table IV. Differential separation of alpha-amylase from proteinase was again independent of the enzyme activities in the original samples. In all cases, less than 0.1% of the original proteinase activity was retained. The per cent of alpha-amylase retained varied from 56 to 66% for malted barleys and from 60 to 74% for the malted wheat flours.

The results obtained from a series of fungal preparations were exceedingly variable. This suggests that the type of organism used and the cultural practices involved may influence the inactivation of alpha-amylase and proteinase. The concentration of accompanying substances also may exert a marked influence on the stability of the enzyme. This was demonstrated by the results from an experiment in which a fungal preparation was diluted with an equal portion of autoclaved malted wheat flour extract, prior to subjecting the preparation to the normal thermal inactivation technic employing low pH. The per cent of alpha-amylase remaining was of the order of 65%, the same as that remaining when a malted wheat flour extract was subjected to the same treatment. The control, using the fungal preparation diluted with an equal portion of water, retained 80% of the alpha-amylase.

It is well to stress, therefore, that the complete inactivation of either alpha-amylase or proteinase cannot be assumed merely because the extract has been subjected to the prescribed conditions recorded in Tables I and III. This is especially true for fungal preparations but to a lesser degree for extracts of malted wheat flour or malted barley. An analysis for enzyme activity should always be made before and after the desired treatment.

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STUDIES ON THE ROLE OF ALPHA-AMYLASE AND PROTEINASE IN BREADMAKING

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ABSTRACT

Alpha-amylase and proteinase supplements prepared by differential inactivation from malted wheat flour and fungal extracts have been utilized to evaluate the role of these enzyme systems in baking using a commercial sponge-dough procedure. Three flours were studied, including commercially milled hard red spring and winter wheat flours and a composite of experimentally milled flour.

Increasing the concentration of alpha-amylase up to 20 times normal concentration increased the loaf volume. The grain and texture was improved with normal concentrations of alpha-amylase but became soft and gummy and lacking in body with higher concentrations. Alpha-amylase caused the crumb to be more compressible.

The effects of increasing the concentration of proteinase differed with the malted wheat flour and the fungal extracts and with the flour employed. Hard red spring wheat flour exhibited a marked response in loaf volume but the winter wheat flour did not respond in this manner. Higher concentrations of proteinase derived from the fungal preparation were detrimental to loaf volume, grain, and texture and decreased crumb compressibility.

Alpha-amylase is largely responsible for the decrease in dough consistency at normal concentrations of a malt supplement. The consistency of dough remained constant when the concentration of alpha-amylase was increased beyond the normal supplementation practice. The effect of the proteinase in decreasing the dough consistency continued with higher con-

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centrations of the supplement. The decrease in dough consistency may be considerable, depending upon the amount of supplement employed and the ratio of alpha-amylase to proteinase activity.

The role of amylase supplements in breadmaking has been investigated extensively (Kneen and Sandstedt, 9; Geddes, 5; and Hildebrand, 6). The attempts to separate and study the effects of each component have failed to be fully conclusive. The recent work of Miller and Johnson (15) and Dirks and Miller (2) on differential separation of alpha-amylase and proteinase in malt supplements provides materials with which the role of these enzyme components in breadmaking may be reinvestigated.

The role of alpha-amylase in increasing the sugar production, and thereby increasing gas production and improving crust color, is commonly accepted (Freeman and Ford, 3). Other roles, however, that alpha-amylase may play are not so clearly demonstrated. Controversy exists as to what component of amylase supplements is responsible for changes in dough consistency, loaf volume, grain and texture, and crumb compressibility. Sherwood and Bailey (21), employing large dosages of malted wheat flour in baking with a straight dough formula, concluded that impaired gas retention resulted from excessive proteolysis. Kozmin (11), working with flour from sprouted grain, demonstrated that the detrimental effects of these flours compared with normal flours was due to excessive splitting of the starch with consequent formation of sticky dextrins, reducing sugars, and releasing water in the crumb.

Miller and Johnson (14) demonstrated that the proteolysis might be excessive in sponge doughs. Salt employed in the straight dough inhibited about 60% of the proteolysis.

Tissue and Bailey (22), Read and Haas (18), Munz and Bailey (17), and Hildebrand and Burkert (7) have employed safranin dye as originally suggested by Marston (12) to precipitate the proteinase from the other enzyme components of malt supplements. Certain conclusions were reached by these authors concerning the role of alpha-amylase and proteinase in baking. Tissue and Bailey (22) concluded that alpha-amylase was responsible for increased loaf volume and improved grain and texture of the baked bread. Munz and Bailey (17) employed a high hydrogen-ion concentration to inactivate the alpha-amylase, and concluded that alpha-amylase was responsible for changes in dough mobility during fermentation. It is possible, however, that the proteinase enzyme may have been inactivated by the citric acid employed in lowering the pH of the extract (Mounfield, 16).

Read and Haas (18) concluded that small quantities of proteases were beneficial with "bucky" type doughs because of the mellowing

effects on the gluten. Excessive dosages of diastatic agents were detrimental because of stickiness imparted to the dough. Hildebrand and Burkert (7) indicated that increased dough mobility was due to alpha-amylase and not to the proteases. Sandstedt, Jolitz, and Blish (19) and Kneen and Sandstedt (10) believed that most of the effects of malt supplements can be explained by the action of alpha-amylase on the starch. These authors did not, however, investigate experimentally the effects of the malt proteinases. Kneen and Sandstedt (10) pointed out that the increase in loaf volume was achieved by increasing the gas retention which was modified as a result of the formation of dextrins in the dough.

Bailey (1) demonstrated that malt extracts produced a softer bread crumb but did not determine what enzyme systems were responsible for this action.

Recent methods (15) of differentially separating the alpha-amylase and proteinase of malt extracts make possible a study of the individual components. Therefore, the object of this study has been to reinvestigate the role of alpha-amylase and proteinase in breadmaking. Included in these studies are the effects of alpha-amylase and proteinase on loaf volume, grain and texture, dough consistency, and crumb compressibility.

Materials and Methods

Three samples of flour representing hard red spring wheat, hard red winter wheat, and a composite of spring and winter wheat were studied. The analytical characteristics of these flours are given in Table I. Two flours were commercially milled bakers patent, bleached

TABLE I
ANALYTICAL DATA FOR FLOURS EMPLOYED IN STUDY
OF MALT SUPPLEMENTATION

Flour type	Protein ¹	Ash ¹	Water ¹ absorption	Dough mix- ing time
	%	%	%	min.
Hard red spring wheat ²	12.3	0.42	68	6.0
Hard red winter wheat ²	11.8	.42	65	4.0
Composite of hard red spring and hard red winter wheat ³	12.7	.44	68	5.0

¹ Expressed on 14% moisture basis.

² Commercially milled.

³ Experimentally milled.

but not malted, and considered typical of the class of wheat which they represent. The third flour was a blend of experimentally milled hard red spring and winter wheats.

The two sources of amylase supplements included a commercial malted wheat flour and a commercial fungal amylase concentrate. These enzyme preparations had a potency of 42.8 and 3300 alpha-amylase units per gram, respectively.

Supplementation was accomplished by extraction of the amylase sources with water or calcium chloride, depending on requisites of the experiment. The alpha-amylase and proteinase were separated differentially by the optimum conditions described by Miller and Johnson (15). In experiments requiring only proteinase, the concentrations of the extract were adjusted to maintain the same proteolysis as would have been provided by the extract before inactivation of the alpha-amylase. Conversely, in experiments requiring only alpha-amylase, the quantity of extract was adjusted to provide the same alpha-amylase activity as would have been provided by the extract before removal of the proteinase.

Adjustment of alpha-amylase was based on the starch dextrinization procedure of Sandstedt, Kneen, and Blish (20). The concentration of extract employed in the dough was based on a relative alpha-amylase activity noted as X^2 . The proteinase activities of the extract were determined by the Ayre-Anderson method as modified by Miller (13).³ In experiments in which either the proteinase or the alpha-amylase was removed differentially the enzymatic activity of each component was determined to ascertain actual activities of the extracts employed in baking.

All baking experiments were conducted on a pilot-plant scale employing a commercial 70% sponge-dough procedure. All malt supplementations were made in the sponge.

The formula was as follows:

<i>Ingredient</i>	<i>Percentage</i>	<i>Sponge</i>		<i>Dough</i>	
		<i>lb.</i>	<i>oz.</i>	<i>lb.</i>	<i>oz.</i>
Flour	100.0	14	—	6.0	—
Yeast	2.0	—	6.4	—	—
Arkady	0.5	—	1.6	—	—
Malt supplement	variable	variable	—	—	—
Sugar	5.0	—	—	1.0	—
Salt	2.0	—	—	—	6.4
Dry skim milk solids	3.0	—	—	—	9.6
Shortening (Crisco)	3.0	—	—	—	9.6
Paniplus	0.25	—	—	—	0.8
Water	variable	variable	—	variable	—

The sponges were mixed for 5 minutes at high speed in a horizontal Day mixer. The sponges were set at 76°–78°F. (24.4°–25.6°C.) and

³ X concentration represents the alpha-amylase activity that would be provided in a dough by 0.25% (based on total flour in dough) of malted wheat flour having an activity of 42.8 alpha-amylase units.

⁴ It is recognized that the method for measurement of proteinase activity based on its ability to hydrolyze hemoglobin is empirical. Experience has shown, however, that the results obtained by this technic are well correlated with changes in dough consistency.

fermented for 4 hours at 80°–82°F. (26.7°–27.8°C.) and 85% relative humidity.

The doughs were remixed to optimum consistency and the dough temperature controlled at 80°–82°F. The doughs were given a 40-minute floor time, scaled to 20 oz., and given 20 minutes' rest before molding. The doughs were proofed at 92°–94°F. (33.3°–34.4°C.) and 90% relative humidity for 50 minutes and baked for 30 minutes at 425°F. (218.8°C.) in a revolving reel type oven. Loaf volumes were determined while the bread was still warm, but grain and texture scores were obtained 18 hours after baking.

The effect of the enzyme components on sponge dough consistency was measured by the farinograph. A portion (530 g.) of the sponge dough after 4 hours fermentation was placed in the large bowl and consistency followed during a 15-minute mixing period. The dough consistency after 15 minutes of mixing was chosen as a measure of enzymatic changes in the dough.

TABLE II

EFFECT OF ALPHA-AMYLASE AND PROTEINASE OF ALPHA-AMYLASE SUPPLEMENTS ON BAKING CHARACTERISTICS OF HARD RED SPRING WHEAT FLOUR

Relative conc. ¹	Malted wheat flour				Fungal amylase preparation			
	Loaf volume	Grain	Texture	Compressibility	Loaf volume	Grain	Texture	Compressibility
	cc.	%	%	g. ³	cc.	%	%	g. ³
CONTROL								
0	2690	80	80	151	2690	80	80	147
1X	2769	85	85	124	2730	80	85	125
5X	2866	80	85	109	2816	90	90	111
20X	2897	60	70	132	2672	60	70	125
SUPPLEMENT MINUS PROTEINASE								
1X	2825	75	85	120	2759	75	75	105
5X	2913	85	85	116	2813	80	85	106
20X	2922	75	75	116	2894	75	75	105
SUPPLEMENT MINUS ALPHA-AMYLASE								
1X ²	2703	75	85	132	2738	80	85	116
5X	2881	85	90	120	2622	75	80	122
20X	2956	70	80	120	2475	60	60	127

¹ X concentration is equivalent to 0.25% of malted wheat flour having an activity of 42.8 alpha-amylase units per gram.

² Concentration adjusted to have proteinase activity equivalent to that in the control.

³ Grams of lead shot required to depress plunger, one inch in diameter, into crumb a distance of 4 mm. Bread stored 66 hours.

The effects of the individual enzyme components on crumb compressibility were followed using the Bloom gelometer. Compressibility of crumb was determined on three loaves from each treatment after 18 and 66 hours of storage in plastic bags sealed with drafting tape. Two readings on each of two slices, one inch thick, cut from the center of the loaf were made, making a total of 12 measurements for each treatment.

Results and Discussion

The effects of supplementation by malted wheat flour and fungal concentrate on baking characteristics and crumb compressibility are summarized in Tables II, III, and IV for hard red spring, winter, and

TABLE III

EFFECT OF ALPHA-AMYLASE AND PROTEINASE OF ALPHA-AMYLASE SUPPLEMENTS ON BAKING CHARACTERISTICS OF HARD RED WINTER WHEAT FLOUR

Relative conc. ¹	Malted wheat flour				Fungal amylase preparation			
	Loaf volume	Grain	Texture	Compressibility	Loaf volume	Grain	Texture	Compressibility
	cc.	%	%	g. ²	cc.	%	%	g. ²
CONTROL								
0	2684	80	80	132	2680	80	80	223
1X	2763	90	90	115	2771	85	85	188
5X	2896	85	70	110	2876	95	95	195
20X	3010	70	70	138	2684	60	60	194
SUPPLEMENT MINUS PROTEINASE ³								
1X	2756	80	85	118	2725	85	85	168
5X	2890	85	80	121	2825	85	85	172
20X	2989	70	70	115	2870	80	80	172
SUPPLEMENT MINUS ALPHA-AMYLASE								
1X ⁴	2870	80	85	121	2779	85	80	184
5X	2808	80	85	124	2533	80	82	227
20X	2792	80	70	123	2584	70	75	231

¹ X concentration is equivalent to 0.25% of malted wheat flour having an activity of 42.8 alpha-amylase units per gram.

² Concentration adjusted to have proteinase activity equivalent to that in the control.

³ Grams of lead shot required to depress plunger, one inch in diameter, into crumb a distance of 4 mm. Bread stored 66 hours.

a composite of spring and winter wheat flours, respectively. The series of various concentrations of the natural enzyme extracts is listed as control. The series containing only alpha-amylase is listed as supplement minus the proteinase. The series containing only pro-

teinase is listed as supplement minus the alpha-amylase. The fungal preparations were adjusted to have an alpha-amylase activity equivalent to that of malted wheat flour, but the proteinase activity of the fungal preparation was approximately 10 times that of the malted wheat flour on an equivalent alpha-amylase basis.

TABLE IV

EFFECT OF ALPHA-AMYLASE AND PROTEINASE OF ALPHA-AMYLASE SUPPLEMENTS ON BAKING CHARACTERISTICS OF A BLEND OF EXPERIMENTALLY MILLED HARD RED SPRING AND WINTER FLOURS

Relative conc. ¹	Malted wheat flour				Fungal amylase preparation			
	Loaf volume	Grain	Texture	Compressibility	Loaf volume	Grain	Texture	Compressibility
	cc.	%	%	g. ³	cc.	%	%	g. ³
CONTROL								
0	2741	90	88	145	2740	90	85	162
1X	2731	92	90	124	2730	95	95	127
5X	2878	80	82	103	2900	88	95	104
20X	2872	75	78	122	2820	60	60	109
SUPPLEMENT MINUS PROTEINASE								
1X	2744	85	80	119	2765	90	90	133
5X	2991	88	85	115	2785	85	85	135
20X	2978	73	75	122	2990	80	80	147
SUPPLEMENT MINUS ALPHA-AMYLASE								
1X ²	2863	85	82	116	2790	88	90	129
5X	2863	95	95	121	2655	80	80	137
20X	2875	82	85	127	2550	50	50	151

¹ X concentration is equivalent to 0.25% of malted wheat flour having an activity of 42.8 alpha-amylase units per gram.

² Concentration adjusted to have proteinase activity equivalent to that in the control.

³ Grams of lead shot required to depress plunger, one inch in diameter, into crumb a distance of 4 mm. Bread stored 66 hours.

Since it was recognized that crumb compressibility might be influenced by loaf volume, the compressibility expressed on a per unit basis is presented in Table V.

The Effects of Natural Enzyme Extract Which Contained Both Alpha-Amylase and Proteinase. Increasing concentrations up to 5X of both malted wheat flour and fungal extracts increased the loaf volume in all samples of flour examined except for the fungal extract which at 20X concentration did not produce a significant change in loaf volume over that of the control. The grain and texture were improved by the use

of a limited amount of either supplements. The improvements in grain and texture included smaller and more uniform cells and a softer, whiter crumb. In nearly all comparisons, optimum grain and texture were obtained with 1X concentration of both malted wheat flour and fungal extract. As the concentration of malted wheat flour extract was increased beyond 1X the grain became more open and the texture became increasingly soft. The texture became particularly

TABLE V
EFFECT OF ALPHA-AMYLASE AND PROTEINASE SUPPLEMENTATION
ON CRUMB COMPRESSIBILITY PER UNIT LOAF VOLUME

Relative conc. ¹	Malted wheat flour			Fungal amylase preparation		
	Control	Minus proteinase	Minus alpha-amylase	Control	Minus proteinase	Minus alpha-amylase
	g./cc.	g./cc.	g./cc.	g./cc.	g./cc.	g./cc.
HARD RED SPRING WHEAT FLOUR						
0	.056			.055		
1X	.045	.042	.049	.046	.038	.042
5X	.038	.040	.042	.039	.038	.047
20X	.046	.040	.041	.047	.036	.051
HARD RED WINTER WHEAT FLOUR						
0	.049			.083		
1X	.042	.043	.042	.068	.062	.066
5X	.038	.042	.044	.068	.061	.069
20X	.046	.038	.044	.072	.060	.090
HARD RED SPRING AND WINTER WHEAT COMPOSITE FLOUR						
0	.053			.059		
1X	.045	.043	.041	.047	.048	.046
5X	.036	.038	.042	.036	.048	.052
20X	.043	.041	.044	.039	.049	.059

¹ See footnote 1 Table IV.

gummy and therefore undesirable at the highest concentrations. The crumb did not become as gummy with the fungal as with the malted wheat flour extract, but became harsh and more coarse. The detrimental effects of high concentrations of malt supplements were more pronounced with the fungal preparation than with the malted wheat flour.

The compressibility of the crumb increased up to 5X concentration, but with 20X the crumb apparently became more firm. The crumb

compressibility per unit volume (Table V) decreased up to 5X concentration but increased when 20X concentration was employed.

Effect of Supplements from Which the Proteinase Was Removed. Increasing the concentration of alpha-amylase extract caused the loaf volume to increase. In some instances of high extract concentration the loaf volume increase was greater than in the instance of the controls containing both alpha-amylase and proteinase. With every flour employing 20X concentration of fungal extract, the absence of proteinase and presence of alpha-amylase caused an increase in the loaf volume. Since sufficient sugar was provided to insure adequate gas production independent of the action of alpha-amylase, it is assumed that the alpha-amylase increased the gas retention. Further experiments showed that gas retention of the *fermenting* dough was not altered. Gas retention of the *baking* dough, however, was improved as indicated by the greater oven response when alpha-amylase was present. These results thus elaborate on the conclusions of Kneen and Sandstedt (10).

Increasing the alpha-amylase derived from either malted wheat flour or fungal concentrate up to 5X concentration improved the grain in nearly all instances. The texture also generally improved up to 5X, but with 20X concentration of alpha-amylase from malted wheat flour the crumb became gummy and moist. The gummy characteristics were not so pronounced with the fungal preparation. This difference between malted wheat flour and fungal alpha-amylase may be due to the lower temperature of thermal inactivation of the fungal alpha-amylase (Johnson and Miller, 8) with consequently less dextrin formation during the baking process. Sticky doughs were not observed at any of the concentrations of alpha-amylase employed. The effects on grain and texture of increasing the alpha-amylase in the absence of the proteinase suggest that alpha-amylase alone may not be so effective in creating improvement as when proteinase is also present in small amounts.

The effects of alpha-amylase on crumb compressibility suggest that this component of malt is mainly responsible for the increased compressibility of crumb. High concentrations of alpha-amylase, however, did not appear to be more effective than lower concentrations (Table V). No consistent difference between the effect of fungal and malted wheat flour amylases could be observed.

Effects of Supplement from Which the Alpha-Amylase Was Removed. Increasing the concentration of proteinase from malted wheat flour caused the loaf volume to increase for the spring wheat and the experimentally milled flours. The loaf volume of the hard winter wheat flour and the experimentally milled flour increased over that of the

controls but did not increase with increasing concentrations of the supplement. Improvement in grain and texture for the hard red spring and experimentally milled flour was observed up to 5X concentration of the supplement. This would suggest that with certain flours the protein may be modified to increase gas retention. These data would appear to support the conclusions of Read and Haas (18).

The effects of increasing concentrations of proteinase derived from the fungal preparation are different from those of malted wheat flour. These differences may be expected when consideration is given to the fact that the fungal preparation had 10 times more proteolytic activity than the malted wheat flour when compared on an equivalent alpha-

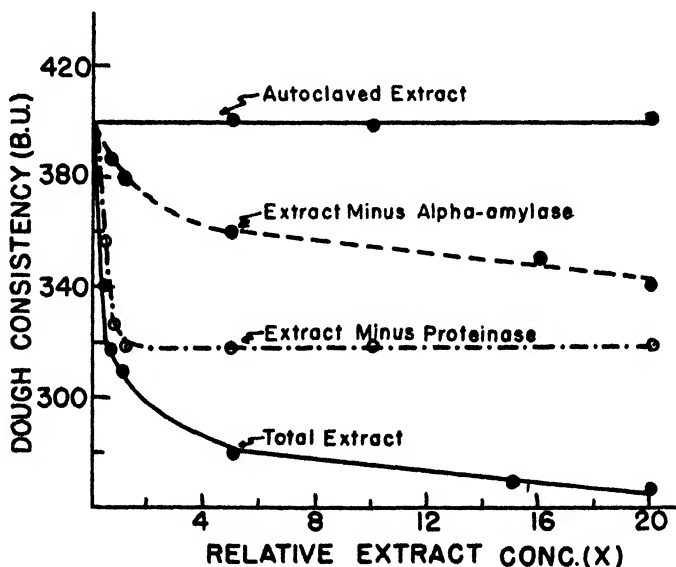


Fig. 1. Effect of normal malted wheat flour extract, extract minus alpha-amylase, extract minus proteinase, and autoclaved extract on dough consistency.

amylase basis. The amount of proteinase supplied by 1X concentration of the fungal extract was generally beneficial, but with increasing amounts the effects were detrimental. Small concentrations of proteinase caused the dough to be more mellow and extensible. Large dosages were detrimental to dough-handling properties, causing sticky and slack doughs. The data obtained from each flour supplemented with the fungal preparation consistently reveal that the presence of both alpha-amylase and proteinase up to 5X concentration improved loaf characteristics. The proteinase at the same concentration caused detrimental effects which were more pronounced in the absence of alpha-amylase. The grain and texture also show that high concentra-

tions (20X) of the proteinase supplied by the fungal extract were more detrimental than that supplied by malted wheat flour.

Small concentrations of proteinase appeared beneficial in increasing the crumb compressibility over that of the controls (Table V), but increasing amounts of fungal proteinase caused increasingly harsh and firm crumb.

Effect of the Enzyme Components of Alpha-Amylase Supplements on Dough Consistency. The effects of the enzyme components of

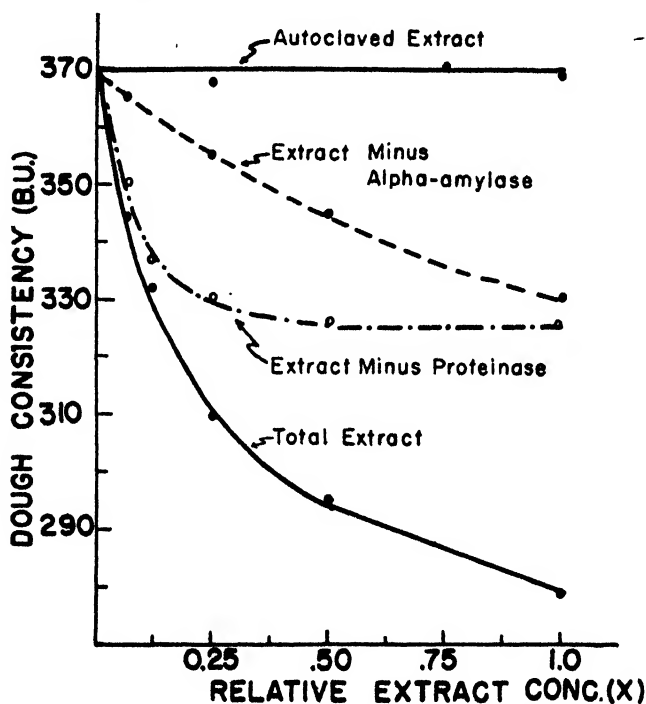


Fig. 2. Effect of normal fungal extract, extract minus alpha-amylase, extract minus proteinase, and autoclaved extract on dough consistency.

malted wheat flour and fungal preparation on dough consistency are shown in Figs. 1 and 2, respectively. That the enzymes are responsible for increased dough mobility is shown by the lack of change in dough consistency when either autoclaved extracts or extracts which had received both enzyme inactivating treatments were employed. The amount of reducing matter in the total extract as determined by iodine titration (Freilich, 4) was found to be insignificant. Sufficient yeast food was added to the sponge to counterbalance the small amount of reducing matter present.

That alpha-amylase is in part responsible for the decrease in dough consistency is evident. Alpha-amylase from both the malted wheat flour and the fungal preparation was responsible for the sharp decrease in dough consistency up to approximately 1X concentration of the enzyme. Further increase in the concentration of alpha-amylase appeared to produce no further change in dough mobility. This suggests that the quantity of starch substrate which is susceptible to attack is limited.

The doughs to which only proteinase was added also decreased in consistency, but not in the same relationship to concentration as the doughs containing alpha-amylase. Dough mobility continued to increase as the concentration of proteinase was increased. It would appear that the protein substrate was not limiting for the proteinase as was the substrate for alpha-amylase.

The effect of increasing the concentration of the total extract produced a curve, representing the combined effects of the proteinase and the alpha-amylase. The presence of both enzyme components was more effective than either component alone in decreasing the dough consistency.

The present study on the components responsible for changes in dough mobility support in part the conclusion of Munz and Bailey (17), Hildebrand and Burkert (7), and Kneen and Sandstedt (10) that alpha-amylase is largely responsible for decreased dough consistency at normal levels of supplementation. The effects of proteinase are significant, however, and cannot be discounted, particularly when an enzyme preparation is employed which has a high ratio of proteinase to alpha-amylase. The mellowing action of increased concentrations of proteinase as illustrated in Figs. 1 and 2 may, at times, be desirable, depending upon the flour employed.

Acknowledgments

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THIAMINE RETENTION IN BREAD AND ROLLS BAKED TO DIFFERENT DEGREES OF BROWNESS¹

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ABSTRACT

Thiamine retention in yeast bread and rolls decreased as the products were baked to a darker crust color, supporting the conclusion that thiamine destruction is increased with increased exposure to heat. Thiamine retention was greater in rolls than in bread, and in clover-leaf and pan rolls than in dinner rolls. The thiamine retention in rolls was greatest in the products having the smallest surface area and the thinnest crust. The greater loss of thiamine in bread than in rolls in these experiments indicates that the length of time a product containing thiamine is exposed to heat is more important in the destruction of the vitamin than the amount of surface exposed to the heat.

The literature on yeast and quick breads reveals that the retention of thiamine is appreciably influenced by the time and temperature of baking. These two factors are so intimately related that it is impossible to study them separately in a product baked to an acceptable doneness. In general, it has been found that a high baking temperature for a short time tends to result in greater retention of thiamine than a lower temperature for a longer time (4). At any given baking temperature, as the length of time increases, the retention of thiamine decreases (11).

It has also been observed that the greatest thiamine destruction occurs in the crust of a loaf of bread, though the loss in the crumb is not insignificant (8, 9, 10, 11). The proportion of crust to crumb is greater in yeast rolls than in bread, but the baking period for the rolls is considerably shorter. A search of the literature at the time this investigation was initiated (1947) revealed no studies on thiamine retention in yeast rolls. It seemed interesting, therefore, to investigate whether the shorter baking period for rolls would compensate for the increased proportion of crust to crumb; also to compare the retentions of thiamine in yeast products baked to a dark rather than a pale crust color (both in an acceptable range).

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Materials and Methods

A total of 15 batches of dough was made. From each batch of dough, bread, pan rolls, clover-leaf rolls, and dinner rolls were baked. Each batch of dough was mixed from the formula given.

<i>Formula for Water Bread and Rolls</i>		<i>Per Cent of Flour</i>
Flour	1090.0 g.	—
Sugar	43.6 g.	4
Salt	21.8 g.	2
Fat	21.8 g.	2
Yeast	32.7 g.	3
Water	660.0 ml. (average) ⁴	60.5

Ingredients. An enriched bread flour was used throughout the experiment. The amounts used were purchased as needed over a period of 8 months. This procedure was used rather than buying the entire lot at the beginning of the study since variation in the thiamine content of flour from bag to bag, even among those bought at the same time from the same mill and stored together until used, has been reported (3). The compressed yeast used in the experiments was purchased as needed in 2-oz. packages. A hydrogenated vegetable fat was used as the shortening. Tap water was used as the liquid.

A Harvard trip balance with a sensitivity of 0.1 g. was used to weigh all ingredients except the flour; the flour and the dough were weighed on a general laboratory torsion balance with a sensitivity of 0.1 g.

Mixing. A straight dough process of mixing was used. The dough was mixed in a Model H Kitchen-Aid mixer; the capacity of the mixing bowl was approximately 4 qt.

Fermentation. The dough was fermented for 75 minutes before molding. Fermentation and proofing of the doughs were carried out in a thermostatically controlled Freas Electric Oven which had been modified for use as a proofing oven and was maintained at a temperature of 30°–32°C. (81°–86°F.) and a humidity of 70% or over.

Molding. For the *bread* a 525-g. portion of dough was weighed and shaped into a loaf following the procedure of the American Association of Cereal Chemists (1). This yielded a loaf of bread weighing approximately 1 lb.

The bread was placed in dull tinned pans of standard shape, the inside top dimensions being 11 cm. × 22 cm. (8½ in. × 4¼ in.), the bottom dimensions, 9 cm. × 20 cm. (8 in. × 3¾ in.) and the depth, 8.4 cm. (3¼ in.).

For the *pan rolls* a 525-g. portion of dough was weighed and formed into a ball. This was manipulated in the same manner as the bread

⁴ The amount of water used varied with the flour absorption; the range was from 630 to 686 ml.

except that instead of forming a square, this portion of the dough was formed into an oblong approximately 5 by 9 inches. It was cut into two approximately equal portions. Each piece was then rolled under the open hands to form a roll approximately 1 inch in diameter and 16 inches in length. Each piece was cut into eight approximately equal portions. Each portion was then rolled into a small ball. The 16 balls of dough were placed side by side in a lightly greased baking pan. This yielded a pan of rolls weighing approximately 1 lb.

For the *clover-leaf rolls* a 262-g. portion of dough was weighed, formed into a ball, and manipulated in essentially the same manner as for the pan rolls, except that the oblong approximated 3 by 8 inches. This was cut into two approximately equal portions and each piece was rolled under the open hands to form a roll about 12 inches in length and $\frac{3}{4}$ inch in diameter. Each roll was then cut into 12 approximately equal portions which were then rolled into smooth balls. Groups of three of these balls were placed into lightly greased muffin pan cups. This yielded eight rolls weighing approximately $\frac{1}{2}$ lb.⁶

For the *dinner rolls* a 262-g. portion of dough was weighed and manipulated in the same manner as for the clover-leaf rolls up to and including dividing the oblong piece of dough. Each piece of dough was then rolled as before to form a roll about 1 by 8 inches. These were cut into four approximately equal portions which were rolled into smooth balls. Four balls were placed in each pan 1.5 cm. ($\frac{5}{8}$ in.) from the side of the pan and with 3.2 cm. ($1\frac{1}{4}$ in.) of space between the balls of dough. This was done to prevent the dough from coming into contact with either the sides of the pan or another ball of dough during proofing. This yielded eight rolls weighing approximately $\frac{1}{2}$ lb.⁶

The pan and dinner rolls were placed in dull tinned pans whose inside top dimensions were 18.6 cm. \times 18.6 cm. ($6\frac{5}{8}$ in. \times $6\frac{5}{8}$ in.); the inside depth was 3.7 cm. ($1\frac{1}{2}$ in.). The clover-leaf rolls were placed in dull tinned muffin pans, the over-all dimensions of which were 26.7 cm. \times 13.8 cm. ($10\frac{1}{2}$ in. \times $5\frac{1}{2}$ in.). The top and bottom radii of the cups were respectively 5.3 cm. ($2\frac{1}{4}$ in.) and 4.5 cm. ($1\frac{3}{4}$ in.); the inside depth was 2.5 cm. (1 in.).

Proofing. All products were placed in the proofing oven as soon as molded. The bread was always molded first; the order of molding the variously shaped rolls was rotated. The products were proofed 1 hour after the last pan was placed in the proofing oven. It took 45 minutes to prepare all of the products for the oven; consequently the

⁶ The limitation of baking facilities made it expedient to use a smaller portion of dough for the clover-leaf and dinner rolls than for the loaf of bread and the pan rolls. Since the variation in thiamine content among the individual clover-leaf rolls and among the dinner rolls had been found to be within the experimental error of the method, this procedure should introduce no significant error.

bread was proofed approximately $1\frac{3}{4}$ hours, and the rolls for varying shorter times. Brooks (4) found that varying the length of time of proofing had no significant effect on thiamine in yeast breads.

Baking. The bread and rolls from a batch of dough were all baked at one time at a temperature of 204.4°C . (400°F .) in a Despatch Electric Rotary Oven, No. 150-R, which maintained the oven temperature within $\pm 2^{\circ}\text{C}$. at the temperature used in the study. Five batches were baked to a pale brown crust color, five to a medium brown color, and five to a dark brown color. The length of the baking period varied as follows:

<i>Series</i>	<i>Color of Product</i>	<i>Baking Time</i>
I Bread	Pale ⁶	30 min.
Rolls	Pale	15 min.
II Bread	Medium Brown ⁶	40 min.
Rolls	Medium Brown	20 min.
III Bread	Dark Brown ⁶	50 min.
Rolls	Dark Brown	25 min.

Cooling. After the products were taken from the oven, they were removed from the pans and placed on wire racks. They were allowed to cool, uncovered, at room temperature for about an hour.

Sampling. All samples were taken in duplicate. A torsion balance with a sensitivity of 0.004 g. was used for weighing the samples for thiamine and moisture determinations.

After a batch of dough was mixed, a small portion was removed to be used for thiamine, pH, and moisture determinations.⁷ For the thiamine determinations, samples of approximately 5 to 6 g. were weighed into 100-ml. glass beakers and covered with watch glasses. Five milliliter beakers were filled with dough for the pH determinations. Seven to eight gram samples for moisture determinations were weighed into aluminum dry-weight pans and tightly covered.

The bread and the pan rolls were weighed and then quartered, the bread according to the method of Brooks (4), and the pan rolls as illustrated in Fig. 1. One quarter of each, to be used for thiamine and moisture determinations, was weighed, sliced, and air-dried. The dried samples were ground and aliquots were taken according to the methods of the American Association of Cereal Chemists (1). A second quarter was used for pH determinations and for subjective judging.

The clover-leaf rolls and the dinner rolls were weighed and individ-

⁶ In each series, the crust color of the bread was somewhat darker than that of the rolls, due to the longer baking period necessary to bake the bread to an acceptable doneness.

⁷ Preliminary work by Brooks (4) showed that samples taken from any portion of the dough gave results comparable with composite samples made up of portions taken from many different parts of the same dough.

uals⁸ chosen for thiamine and moisture determinations according to the diagram in Fig. 1. The selected rolls were sliced, air-dried, and sampled in the same manner as the bread. The remaining rolls were used for pH determinations and subjective judging.

Moisture Determinations. Moisture determinations on both dough and baked products were carried out according to the methods of the American Association of Cereal Chemists (1).

pH Determinations. A Beckman glass-electrode pH meter, laboratory model, was used for all pH determinations. The pH determinations on the dough were made by introducing the electrodes directly into the dough sample. In testing the crumb of fresh bread and rolls, a piece approximately $\frac{1}{2} \times 1\frac{1}{2}$ inches from the soft center

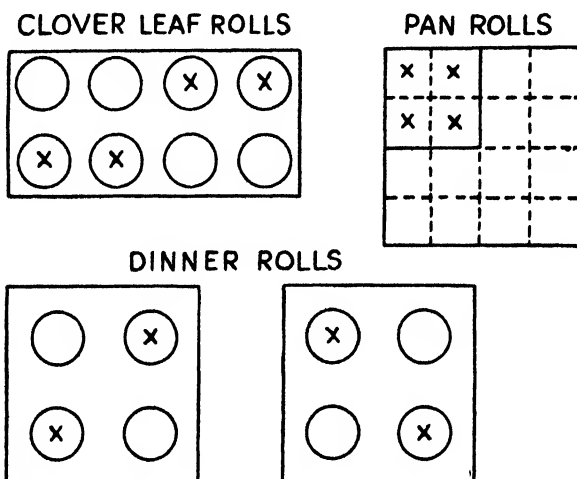


Fig. 1. Design for selecting samples of pan, clover-leaf, and dinner rolls for thiamine, pH, and moisture determinations and for subjective judging. X = rolls chosen for thiamine determinations.

was wrapped around the electrodes and held in a 5-ml. beaker while the determination was made.

Thiamine Determinations. Thiamine determinations were made on the dough and on the air-dried samples of the baked products by a procedure essentially that of Conner and Straub (5).

Expression of Results. The thiamine content of the bread and of the rolls was calculated as micrograms per gram of fresh baked product; and the percentage thiamine retention, on the basis of the total weight and total thiamine content of the products before and after baking. These methods do not require the use of dry weight data, thus eliminating any experimental errors inherent in these determina-

⁸ Preliminary investigation showed that there was little variation in the thiamine content of the four quarters of a pan of pan rolls and that the thiamine content of the individual rolls from one batch of clover-leaf rolls or of dinner rolls did not vary more than 10%.

tions. They also take into account the differences in moisture content between the raw and baked product and so show the actual change in thiamine content during baking. The use of the total change method in expressing thiamine retention values was recommended by Cooperman and Elvehjem (6). This method was used by workers of the Bureau of Human Nutrition and Home Economics in reporting studies on yeast breads (12).

Results and Discussion

Dough. For the 15 batches of dough the average moisture content was 45% and the average pH 5.7. The average thiamine content of the moist doughs was found to be 2.8 μ g. per gram, which is within the range generally reported in the literature.

TABLE I
AVERAGE WEIGHT LOSS IN YEAST BREAD AND ROLLS BAKED
TO DIFFERENT DEGREES OF BROWNESS¹

Crust color	Bread %	Pan rolls %	Clover-leaf rolls %	Dinner rolls %
Pale	11.35 \pm 0.30 ²	8.46 \pm 0.10	15.57 \pm 0.47	16.11 \pm 0.22
Medium	13.90 \pm 0.29	10.36 \pm 0.16	18.17 \pm 0.77	19.39 \pm 0.86
Dark	16.95 \pm 0.27	12.23 \pm 0.47	22.14 \pm 0.92	24.43 \pm 0.62

¹ Based on the weight of the raw, molded dough.

² Standard error of the mean.

Bread and Rolls. All of the breads and rolls were rated "acceptable" on judging. In all cases the weight of the baked products decreased with the length of the baking period; weight loss was also influenced by the shape and size of the products (see Table I), the greatest loss occurring in the individually baked rolls. The pH of the

TABLE II
THIAMINE RETENTION IN YEAST BREAD AND ROLLS BAKED TO DIFFERENT
DEGREES OF BROWNESS DETERMINED BY ASSAYING THE TOTAL PRODUCT

Crust color	Bread %	Dinner rolls %	Clover-leaf rolls %	Pan rolls %
Pale	83.1 \pm 1.9 ¹	89.5 \pm 1.1	93.0 \pm 1.5	92.6 \pm 0.7
Medium	80.4 \pm 2.1	84.5 \pm 1.2	89.8 \pm 2.0	91.5 \pm 2.1
Dark	73.8 \pm 1.5	77.5 \pm 0.6	84.0 \pm 1.0	87.8 \pm 0.8

¹ Standard error of the mean.

crumb of the baked products was not affected by any of the variables studied. The average per cent of thiamine retained in the breads and rolls baked to different crust colors, based on the total weight² of the dough, is presented in Table II and in Fig. 2. The average

² The per cent thiamine retention varies slightly with the method of calculation. The one used is considered the most reliable.

thiamine content, expressed as micrograms per gram of the fresh baked product, is reported in Table III.

Thiamine Retention. As would be predicted, in all products the retention of thiamine decreased as the crust color deepened owing to the increase in the baking time. The differences in thiamine destruction as a result of baking from a pale to a medium crust color, and from a pale or medium to a dark crust color, are significant at odds of 99:1. The percentage loss of thiamine was greater as a result of baking from a medium to a dark crust color than as a result of baking from a pale

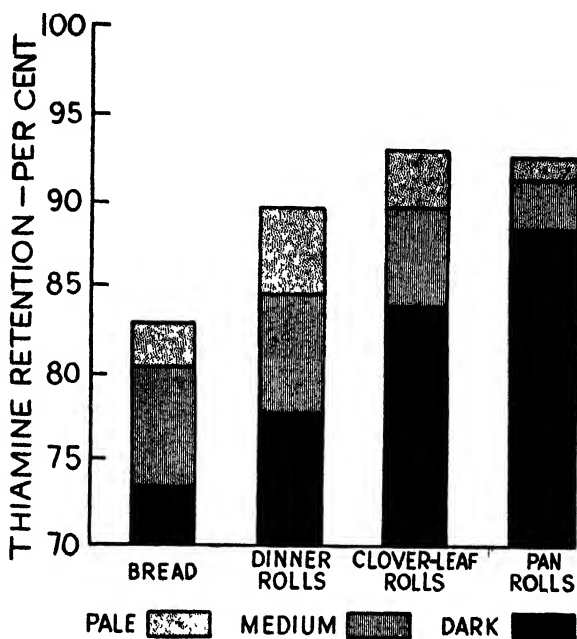


Fig. 2. Thiamine retention in yeast bread and rolls determined by assaying the total product.

to a medium crust color, indicating that thiamine destruction occurred more rapidly as the baking time was increased.

The loss of thiamine was also influenced by the shape and size of the products. In each series the retention of thiamine was greater in rolls than in bread. This finding is in general agreement with the results of studies on the thiamine retention in bread and rolls reported recently by the Bureau of Human Nutrition and Home Economics (12). They obtained retentions of 69.4 to 70.3% and 65.9% for rolls and bread respectively. Their absolute values are, however, considerably lower than those reported in this study. Since formula, baking times, and baking temperatures were different in the two studies, it is

difficult to compare the results. Comparison is further complicated by the lack of information on the shape and size of the rolls in the study by the Bureau.

Among the rolls prepared in this study the retention of thiamine was least in the dinner rolls. The vitamin retention was similar in the clover-leaf and pan rolls, although, with the exception of products baked to a pale crust color, the greatest average retention was always observed in the pan rolls. By analysis of variance the difference in thiamine retention is found to be highly significant when comparing bread and rolls, and also when comparing dinner rolls with pan and clover-leaf rolls. The difference in thiamine retention between pan and clover-leaf rolls is not significant.

Thiamine destruction has been reported as directly related to the amount of crust in baked products (2, 8, 9, 10, 11). The results of this study confirm this view. In rolls the proportion of crust to crumb varies with the surface area exposed to heat and with the thickness of the

TABLE III
AVERAGE THIAMINE CONTENT OF FRESH YEAST BREAD AND ROLLS
BAKED TO DIFFERENT DEGREES OF BROWNESS

Crust color	Bread μg./g.	Pan rolls μg./g.	Dinner rolls μg./g.	Clover-leaf rolls μg./g.
Pale	2.67±0.08 ¹	2.87±0.04	3.03±0.06	3.13±0.05
Medium	2.57±0.08	2.81±0.08	2.89±0.12	3.02±0.10
Dark	2.45±0.04	2.76±0.04	2.83±0.03	2.98±0.04

¹ Standard error of the mean.

crust, both factors influenced by the type of roll. The pan rolls, in which the thiamine retention was high, had the smallest proportion of crust to crumb; the dinner rolls, in which the thiamine retention was lowest, had the greatest proportion of crust. Since the surface area exposed to heat was similar in clover-leaf and dinner rolls, it might be expected that the thiamine retention would be similar. It is believed that the greater retention in the clover-leaf rolls can be explained by the difference in the character of the crust; in the clover-leaf rolls, the crust in contact with the baking pan was thinner than that exposed to the direct heat.

Although the surface area exposed to heat would be less in a loaf of bread than in an equivalent amount of rolls, the crust of the bread was in every series rated by the judges as thicker than that of the rolls. This could account for the relatively large loss of thiamine in bread as compared with rolls. In the toasting of bread, it has been observed that the destruction of thiamine is due to the penetration of heat into the slice of bread as well as to the length of time of the toasting process

(7). Apparently the shorter exposure to heat required in baking yeast rolls compensates for the increased surface area of the rolls compared with a loaf of bread.

Thiamine Content. From Table III it will be observed that the thiamine content per gram of the various products decreased as the crust color deepened; also that the amount of thiamine was greater in rolls than in bread. Among the different types of rolls the highest content was found in the clover-leaf rolls. The higher content in dinner rolls than in pan rolls is undoubtedly a reflection of the lower moisture content of the dinner rolls. Although these differences in thiamine are small they are found by analysis of variance to be significant, indicating that the treatments accorded the samples were responsible for the differences observed.

While the findings of this study are of theoretical interest, their practical significance in the average diet is limited. Even using the extreme values, only about 10% more of the National Research Council daily recommended allowance of thiamine for a moderately active man would be furnished by six clover-leaf rolls baked to a pale brown color than by six slices of bread, made from an equal amount of dough, baked to a dark brown crust color, i.e. 36.5% of this allowance versus 25.6%.

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LIPOLYSIS IN DOUGHS MADE FROM VARIOUS CEREAL FLOURS¹

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ABSTRACT

The relative lipase activity of cereals and factors influencing lipolysis were investigated by following the increase in fat acidity of doughs containing the cereal flour under study, margarine, sucrose, and water. Flours milled from oat groats exhibited a much higher lipase activity than those made from wheat, rye, and barley. Germination of rye and oats for four days at 20° to 25°C. did not increase their lipase activity under the experimental conditions employed.

Mixtures of wheat flour and of oat flour commercially milled from oat groats prepared by the usual drying or roasting procedure prior to dehulling gave doughs which developed a soapy taste when their fat content was high. In contrast, this unpleasant phenomenon did not occur when the oat flour was milled from oat groats prepared by a commercial wet-dehulling procedure which involved washing, heat conditioning, and removal of the hulls in a centrifugal hulling machine. Laboratory experiments showed that the thermal inactivation of oat lipase is much greater when the heat treatment is carried out at 20% than at 12% moisture. That the development of a soapy taste in doughs containing certain oat flours is due to the high lipase activity of these flours, rather than to the nature of the shortening used in the baking formula, is further substantiated by the fact that several commercial fats were all strongly hydrolyzed in doughs containing unheated oat flour.

The shortage of wheat in Sweden during and since World War II made it necessary to mill mixtures of wheat with other cereals, rye, barley, and oats, to obtain sufficient flour. The addition of these cereals to wheat had a deleterious effect upon the strength and color of the flour. In addition, the flour produced by certain mills from mixtures which contained oats gave a very unpleasant soaplike taste to baked products made from doughs with a high fat content. This bad taste was most pronounced when the dough was kept for a long time, as is common in baking certain types of cakes. The soapy taste would appear to be the result of lipolytic action in the dough and the present investigation was undertaken to elucidate its cause.

The limited knowledge of the role of lipases in milling and baking has recently been reviewed by Sullivan (5). The occurrence of lipases in plants was first described in 1871 by Müntz (3), and several years

¹ Manuscript received October 4, 1948.

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later Fokin (2) found wide variations in the lipase content of different oil seeds. Sullivan and Howe (6) investigated the lipases of wheat, their distribution in different wheat products, and the effect of germination on lipase activity. Bamann and Ulmann (1) investigated the lipase content and pH optimum for a number of plants and plant tissues; they found the relative lipase content of oats to be 5, barley 4, and wheat very little. Secchi (4) studied the lipase activity of different wheat varieties, the temperature and pH optima for lipase, and the role of lipolysis in macaroni doughs. He found that heating the doughs at 60°C. for 20 minutes destroyed all the lipase and that the lipase activity was greatly diminished when the flour or semolina used in the doughs was rancid.

In the present study the following factors were investigated: (1) the relative lipase activities of flours milled from wheat, rye, barley, oats, and corn; (2) the influence of germination of rye and oats in the lipase activity of flours milled from them; (3) the influence of heat treatment of oats on the lipase activity of oat flour; and (4) the relative rate of lipolysis of different fats by oat flour.

Lipase Activity of Different Cereals

The relative lipase activity of flours prepared from different cereals was studied in doughs prepared by mixing thoroughly 20 g. of the particular flour under study, 10 g. of bakers' margarine, 10 g. of sucrose, and 2 g. of water. Dough samples weighing 2 g. were placed in wide test tubes which were inserted in a thermostat maintained at 20°C.³ At appropriate intervals of time, 40 ml. of diethyl ether were stirred into the dough to extract the fatty acids and stop the reaction.⁴ After filtration, 20 ml. of 96% ethanol were added and the solution was titrated with 0.05 *N* sodium hydroxide employing phenolphthalein as the indicator.

The results recorded in Table I show that the increase in fat acidity of the doughs made with wheat, rye, barley, and corn flour was very small but was rather high with oat flour. After 24 hours the doughs made from oat flour had a very strong soaplike smell and taste.

Effect of Germination on Lipolysis Produced by Rye and Oats

Rye and oat grains were soaked in water overnight and germinated between sheets of wet filter paper at 20° to 25°C. for four days; at the

³ The buffer capacity of the doughs prevented any appreciable change in pH during lipolysis. For example, the pH of wheat flour dough after digestion for 0.25 hour was 5.80 and only decreased to 5.71 after 57 hours digestion; the corresponding pH values for oat flour dough were 5.60 and 5.45.

⁴ That diethyl ether effectively extracted the fatty acids was shown by determining the acidity of the extract and of the flour-sugar residue after various intervals of digestion; while the acidity of the ether extract increased with time, that of the residue remained low and virtually constant. As constant values were obtained when aliquots of the same ether extract were titrated after standing for various periods of time, lipolysis was completely inhibited by the diethyl ether.

TABLE I
RELATIVE LIPASE ACTIVITY OF CEREAL FLOURS¹
(Activity determined in doughs at 20°C.)

Flour	Fat acidity ²					
	Digestion time in days					
	0	1	2	3	4	5
Wheat	0.2	0.2	—	0.3	0.3	—
Rye	0.3	0.3	0.3	0.5	—	0.5
Barley	0.5	0.6	0.4	0.5	—	0.5
Oats (dehulled)	0.5	5.0	—	8.7	9.0	—
Corn	0.6	0.5	0.4	0.5	—	0.5

¹ Approximate extractions for the flours prepared from the different cereals were: wheat, 60%; rye, 50%; barley, 60–65%; corn, 60%; and oats (dehulled) 35–40%.

² Fat acidity is expressed as ml. of 0.05 *N* NaOH required to neutralize the fatty acids in 2 g. of dough.

end of this time the sprouts had grown to at least the length of the grain. The sprouted grain was dried at room temperature, milled into flour, and the lipase activity determined in doughs prepared in the manner described in the previous section but maintained at 37°C.

The results in Table II indicate that germination did not increase the lipase activity of either oats or rye. This is very surprising as Sullivan and Howe (6) found that when wheat was germinated, the lipolytic activity increased for triglycerides of higher fatty acids but not for those of the lower fatty acids.

Influence of Heat Treatment of Oats on Lipase Activity of Oat Flour

Doughs made with oat flours from some mills developed a soapy taste while those from other mills did not exhibit this characteristic.

TABLE II
EFFECT OF GERMINATION ON LIPASE ACTIVITY OF OATS AND RYE
(Activity determined in doughs at 37°C.)

Nature of sample	Fat acidity ¹				
	Digestion time in hours				
	0.25	1	3	5	24
Oats, control	1.8	2.5	4.0	5.7	11.8
Oats, germinated	1.4	1.8	3.5	5.1	11.1
Rye, control	0.3	0.4	0.3	0.4	0.5
Rye, germinated	0.3	0.6	0.4	0.5	0.7

¹ Fat acidity is expressed as ml. of 0.05 *N* NaOH required to neutralize the fatty acids in 2 g. of dough.

There was reason to believe that this difference in behavior depended on the fact that the oats had been treated differently in preparing them for milling. Comparison of the increase in fat acidity of doughs made with oat flour supplied by three mills, given in Table III, shows that the flour supplied by the TK mill was much the lowest in lipase activity and that the difference could be readily detected in doughs containing only 10% of the respective oat flours. The lipase activity of oat flours from the same mill may vary rather widely, and it seemed that this might possibly be due to varietal differences. However, Swedish white and black oats, Danish white oats, and American white oats all

TABLE III

RELATIVE LIPASE ACTIVITY OF OAT FLOURS SUPPLIED BY DIFFERENT MILLS
(Activity determined in doughs at 20°C.)

Mill	Fat acidity ¹				
	Digestion time in days				
	0	1	2	3	6
Doughs made with oat flour					
TK	0.3	0.4	0.3	0.4	0.7
SV	0.9	2.7	4.0	4.9	7.4
ST	0.7	2.0	3.1	—	4.0
Doughs made with 90% of wheat flour and 10% of oat flour					
TK	0.3	0.3	0.3	0.3	0.3
SV	0.4	0.6	0.8	1.0	1.6
ST	—	1.1	—	2.0	—

¹ Fat acidity is expressed as ml. of 0.05 N NaOH required to neutralize the fatty acids in 2 g. of dough.

had practically the same lipase activity, and it is therefore more likely that the variations in lipase activity between and within mills are due to differences in the moisture content of the oats during heat treatment and in the temperature and time of heat treatment. The usual system for dehulling oats is first to subject them to a drying or slow roasting process to reduce the moisture content to 5–7% (original moisture 12–18%) after which they are dehulled between stones. In the Tre Konor mill a wet dehulling system is employed in which the oats are washed, heat-conditioned at a moisture content of 25–30%, and the hulls removed in a centrifugal hulling machine. The oat groats are then dried for 7 hours at 70°C., which lowers their moisture content to

TABLE IV
EFFECT OF HEAT TREATMENT OF OATS ON LIPASE ACTIVITY OF OAT FLOUR
 (Activity determined in doughs at 37°C.)

Treatment	Fat acidity ¹				
	Digestion time in hours				
	0.25	1	3	5	24
Control, unheated	1.8	2.5	4.0	5.7	11.8
Heated at 12% moisture	1.0	1.6	2.9	3.5	9.4
Heated at 20% moisture	0.5	0.5	0.6	0.9	1.6

¹ Fat acidity is expressed as ml. of 0.05 N NaOH required to neutralize the fatty acids in 2 g. of dough.

8-9%. It seems probable that this method of dehulling will result in a greater destruction of lipases than the customary method.

To test the effect of moisture content on thermal inactivation of lipase, subsamples from one lot of oats were conditioned to 12% and 20% moisture and heat-treated in closed containers at 70°C. for 12 hours. The lipase activity of oat flour prepared from the samples was determined in doughs at 37°C. in the manner already described. The results in Table IV show that the thermal inactivation of lipase is greater when the heat treatment is carried out at 20% instead of 12% moisture. They support the opinion of the authors that the wet-hulling of oats is superior to the customary dry-hulling procedure when they are to be used to produce oat flour for mixing with wheat flour.

TABLE V
RATE OF LIPOLYSIS OF DIFFERENT COMMERCIAL FATS BY OAT FLOUR
 (Activity determined in doughs at 37°C.)

Nature of fat	Fat acidity ¹				
	Digestion time in hours				
	0.25	1	3	5	24
Butter	0.9	2.2	2.5	4.9	12.9
Household margarine ²	0.9	1.6	3.9	4.6	12.2
Bakery margarine ³	0.5	2.2	4.4	6.2	11.8
Salad oil	0.6	1.6	4.3	5.0	13.0

¹ Fat acidity is expressed as ml. of 0.05 N NaOH required to neutralize the fatty acids in 2 g. of dough.

² Household margarine contained 45% of cocoa fat, 20% of rape seed oil, and 35% of hydrogenated whale oil.

³ Bakery margarine was composed of 20% of rape seed oil, 30% of lard and tallow, and 50% of hydrogenated whale oil.

Rate of Lipolysis of Different Commercial Fats by Oat Flour

When the soapy taste in bread was first encountered, some thought that it was due to the nature of the shortening. Accordingly the relative susceptibility of different fats commonly used in Sweden to the lipase of unheated oat flour was investigated. The results in Table V show that all of the fats underwent extensive lipolysis, although the smell and taste of the doughs were different because of variations in the nature of the fatty acids which were liberated. These results support the view that the unpleasant taste experienced when certain oat flours are used in doughs is due to their high lipase activity.

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RELATION BETWEEN AMYLASE ACTIVITIES OF MALTED WHEATS AND MALTED WHEAT FLOURS¹

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ABSTRACT

Malted wheats were readily milled on a two-stand micro experimental mill to a 95% patent flour representing a 50% yield by tempering the wheat to 13.5% moisture and employing a simple system of three breaks and four reductions. Differences in the amylase activity of the flour from replicate millings of one lot of malted wheat were statistically significant. The amylase activities of 30 malted wheats of widely varying activity bore a curvilinear relation to the activities of the flours milled from them, as determined by measuring the stimulation in dough gas production of a common base flour produced by 0.25% additions of each malt supplement. When amylase activity was measured in terms of the reciprocal of the dosage of the sample required to produce a given stimulation in gas production, there was

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a linear relation between the corresponding values for malted wheat and malted wheat flours. The high correlation between these variables, $r = +0.984$, makes it possible to estimate the activity of the flours from the data for wheats with high accuracy, thus eliminating the labor and error involved in experimental milling.

In preparation for a further investigation of the effects of wheat variety, environment, and malting conditions on the properties of wheat malt (2), the present study was undertaken in 1942 to determine whether the value of malted wheat flours for breadmaking could be predicted from determinations on the malted wheats, thereby eliminating the labor and errors involved in experimental milling. At that time cereal chemical laboratories commonly evaluated malted wheat flour for breadmaking purposes by determining either the quantities required to give a definite flour maltose value or dough gas production (3, 7, 8). Hildebrand and Geddes (6) found that for any given malt the stimulation in gas production varied directly as the logarithm of the dosage, whereas the relationship between dosage and the increase in flour maltose value was best represented by a quadratic equation. The estimation of malt activity was thus more directly and simply made from gas production data than from flour maltose values. In applying the gas production technique they ascertained the dosage of malted wheat flour required to produce a given stimulation in gas production and expressed the activity in terms of the reciprocal of the required dosage.

This technique requires the addition of several levels of the malted wheat flour to a common base flour and, for greatest accuracy, it is necessary to estimate the dosage needed to produce the selected response from regression equations for the relation between malt flour dosage and gas production. Meredith, Eva, and Anderson (9) used a much less laborious technique in evaluating experimental malted wheat flours. This consisted in measuring the increase in gas production of doughs containing a standard flour which resulted from the addition of a fixed weight of malt flour.

In the present study, the amylase activities of ground malted wheats of widely varying activity were determined by the techniques of Hildebrand and Geddes (6) and of Meredith, Eva, and Anderson (9) and the values compared with those for the corresponding malted wheat flours produced by a simple experimental micro-milling procedure.

Materials and Methods

Wheat Malts. Thirty wheat malts, experimentally produced from soft white and hard red spring wheat during the course of an investigation by Dickson and Geddes (2), were employed. Fifteen malts were made from each wheat representing the combination of three steeping

levels (35, 40, and 45% moisture) and five germination times (2 to 6 days). These samples thus represented a wide range in amylase activity.

Milling. The milling was carried out in a two-stand micro mill similar to that described by Geddes and Frisell (4). Eighty-gram samples of each wheat malt were tempered to 12.5% moisture and allowed to stand for 16 to 18 hours; 30 minutes before milling, the moisture content was raised to 13.5%. Three breaks and four reductions were made with the separation of bran, shorts, 95% patent, and clear flour employing No. 18 wire, No. 64 GG, and No. 9 XX sieves. These three breaks were made without unloading the sieves which were shaken for 30 seconds after each break. The overs of the No. 18 wire sieve were discarded as bran. The middlings on No. 64 GG were reduced twice and the overs were discarded as shorts. The stock on the 9 XX silk was reduced twice and the overs weighed as clear flour. The throughs of each reduction were weighed separately and sufficient flour from the last reduction was added to that previously milled to obtain a 95% patent flour.

To secure an index of the replicability of the milling technique in respect to the amylase activity of the resulting flours, six samples from a bulk lot of malted wheat were milled by the foregoing procedure.

Amylase Activity. The amylase activities of the experimentally malted wheats and the corresponding flours were estimated in two ways, namely, by the technique of Davis and Tremain (1) as modified by Hildebrand and Geddes (6), and by making 0.25% additions of each to a common base flour of low gassing power and measuring the gas production of doughs prepared from them. The amylase activity of the flours obtained in the replicate millings of the commercial sample of malted wheat was determined only by the latter procedure. The malted wheats were ground in a laboratory model Wiley mill to pass a sieve having round holes 0.5 mm. in diameter. Moistures were determined on the ground wheats and flours and all dosages adjusted to a constant moisture basis. Duplicate doughs were made with 14 g. of each flour (including malt additions) and 10 ml. of yeast suspension containing 0.42 g. of compressed bakers' yeast; the gas production at 30°C. was measured over a 5-hour period.

Results and Discussion

Milling. Malting has such a pronounced mellowing effect that the malted wheats were very readily milled to an approximately 50% yield of 95% patent flour on the short system employed. For the six replicate millings of the commercial lot of malted wheat the standard error of patent flour yield was 0.74%. The mean gas production of

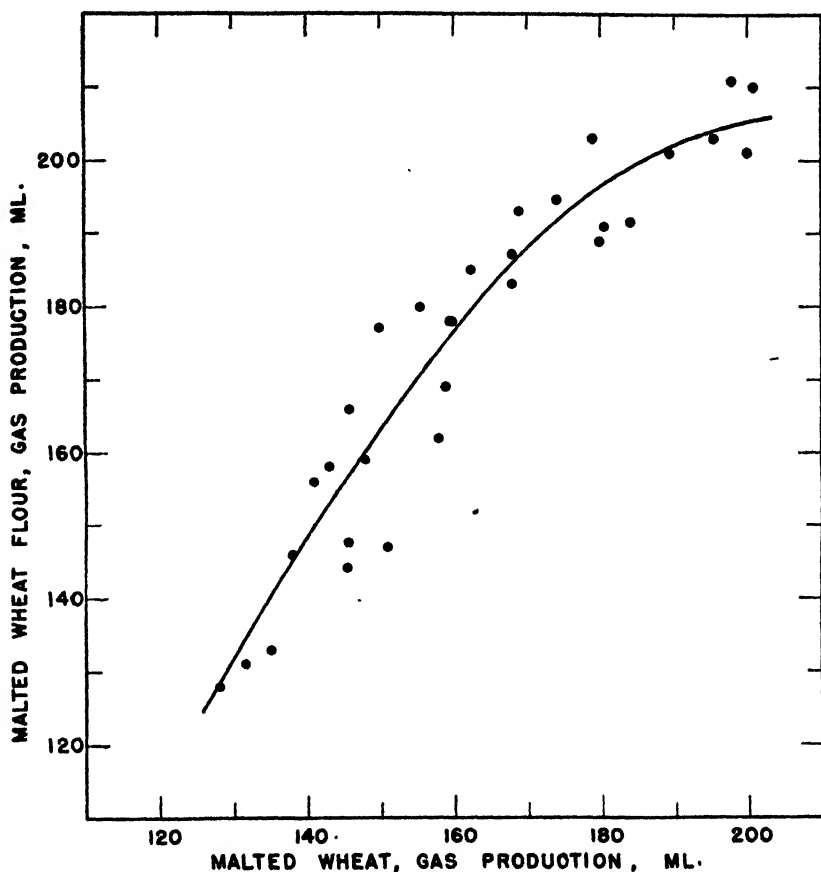


Fig. 1. Relation between the gas production of doughs supplemented with 0.25% additions of ground malted wheats and malted wheat flours milled therefrom. The curve was fitted by the method of least squares.

doughs containing 0.25% additions of these malted wheat flours varied from 198.6 to 202.7 ml. and a variance analysis showed that the differences due to milling were statistically significant.

Amylase Activity. The relation between the gas production of doughs supplemented with 0.25% additions of ground malted wheats and malted wheat flours, respectively, is shown in Fig. 1 and by the following statistical constants:

<i>Statistics for gas production (30 pairs)</i>	<i>Amylase supplement</i>	
	<i>Malted wheat x</i>	<i>Malted wheat flour y</i>
Mean, ml.	162.8	174.2
Standard deviation, ml.	21.5	24.2
Coefficient of variability, %	13.2	13.9

While the correlation based on the assumption of a linear relationship is quite high ($r = +0.924$, 1% pt. = 0.463), an analysis of variance (10) showed that the deviations from linear regression were significant and that the data were best represented by the second degree polynomial: $y = a + bx + cx^2$ where y and x represent the activities of malted wheat flour and malted wheat respectively and a , b , and c are constants. When allowance was made for curvilinearity of regression,

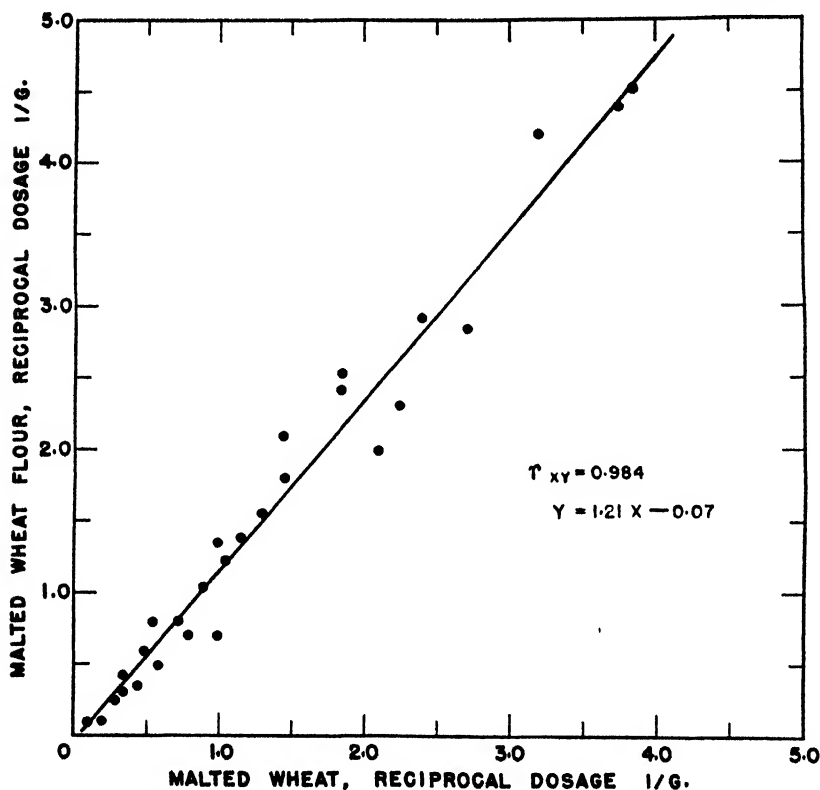


Fig. 2. Relation between the amylase activities of malted wheat and the flour milled therefrom, when the activity is measured in terms of the reciprocal of the dosage (grams supplement per 100 g. flour) required to produce a constant gas production in a wheat flour dough.

the total correlation between the gas production values was increased to $r = +0.976$.

The flours are more active than the malted wheats. When the gas production of the doughs supplemented with the wheats exceeds about 180 ml., the activities of the wheats do not appear to be as widely differentiated as those of the flours.

The amylase activities of the malted wheats and corresponding flours, as expressed by the reciprocal of the dosage in per cent required

to raise the 5-hour gas production for a flour of low gassing power to 205.0 ml., are plotted in Fig. 2. The data were analyzed statistically and certain of the pertinent constants follow:

<i>Statistics for reciprocal dosage (29 pairs)</i>	<i>Amylase supplement</i>	
	<i>Malted wheat</i> x	<i>Malted wheat flour</i> y
Mean amylase activity, 1/g.	1.325	1.532
Standard deviation, 1/g.	1.060	1.300
Correlation, r_{xy}	+0.984	
Regression equation	$Y = 1.207 x - 0.067$	

The regression between the amylase activities of malted wheats and their flours is linear and the high correlation makes it possible to estimate the activity of the flours from the data for wheats with high accuracy. In this series the standard error of prediction is ± 0.17 reciprocal dosage units.

This investigation confirms observations in the literature that the use of a single dosage of an amylase supplement in gas production tests is not a particularly desirable procedure for measuring the relative amylase activity of a series of samples (1, 5, 6). As the quantity of amylase added is increased either by using larger dosages of a given malt or by adding the same quantities of malts of varying activity, the stimulation in gas production becomes less and less with each increment of amylase added, that is, the analytical values obtained by such measures bear a curvilinear relation to the activity of the sample. Even though the curvilinear relationship may be quantitatively defined, it appears that the evaluation of high activities would be somewhat less precise by the single dosage method than by a method which gives a linear relationship.

When the amylase activity is estimated by ascertaining the reciprocal dosages required to produce a given stimulation in gas production, the relation between the values for malted wheats and flours is linear and of a high order. Determinations of the activity of malted wheats by this technique will serve as a convenient and reliable measure of their value for the production of malted wheat flours for use in breadmaking. Analyses of the ground malted wheats eliminate the labor and errors involved in experimental milling.

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EFFECT OF WHEAT CLASS AND GERMINATION MOISTURE AND TIME ON MALT YIELD AND AMYLASE ACTIVITY OF MALTED WHEAT¹

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ABSTRACT

Composite samples of grades one and two hard red spring, hard red winter, soft red winter, and soft white wheats representing marketings of these samples and grades for the 1940 and 1941 crops, respectively, were each malted in duplicate at three moisture levels (approximately 35, 40, and 45%) and five germination times (2, 3, 4, 5, and 6 days) at 16°C. and data obtained for malting loss, cleaning loss, and amylase activity.

Malting loss (which comprised the losses in dry matter due to leaching in the steep water, respiration, and the removal of rootlets and sprouts in cleaning the dried malt) was influenced significantly by wheat class, year, moisture, and time of malting. Over-all moisture levels and germination periods, the wheat classes ranked in order of increasing mean malting loss for each year were: soft red winter, hard red spring, hard red winter, and soft white wheat. The malting losses increased when the wheats were malted at higher moistures or for longer times.

Cleaning loss, which represented the loss in dry matter by the removal of rootlets and sprouts from the dried malts and is a rough measure of growth, was influenced by the same factors as malting loss of which it forms a part. The increase in cleaning loss with increased germination time varied rather

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widely and was relatively small at the 35% moisture level which was too low for good root and sprout growth.

The relative amylase activities of the malts, measured in terms of the reciprocal of the malt dosage required to give a desired level of gas production in a fermenting dough, was influenced significantly by wheat class and especially by the moisture and time of malting. For the two years and over-all malting conditions, soft white wheat yielded malt of the highest activity, followed by soft red winter, hard red winter, and hard red spring wheat in the order named. With an increase in the moisture content at which the samples were germinated, there was a greater increase in the amylase activity of the malts prepared from soft white and soft red winter wheats than of those from the hard red spring and hard red winter wheats.

Malting loss and amylase activity were highly and significantly correlated ($r = +0.710$). The relative influence of germination moisture on these variables indicates the use of moisture levels around 40% as the most economical, since the increased loss in malting at 45% was not compensated by a proportional increase in amylase activity.

The production of malted wheat flour for use in breadmaking has assumed wide commercial importance, but there have been few published studies on the effects of wheat type, environment, and malting conditions on the properties of wheat malt. In a preliminary study, Geddes, Hildebrand, and Anderson (2) investigated the effect of two levels of protein content, germination moisture, germination time, and kilning temperature on the value of malt prepared from hard red spring and amber durum wheat. Amylase activity, expressed as the reciprocal of the amount of malted wheat flour (experimentally milled from the malts) required to produce constant gassing power in blends with a common base flour, was influenced by all factors except kilning. Increasing the germination moisture from 40 to 44% and the germination time at 54°F. from 3 to 5 days and the use of hard red spring wheat rather than durum raised the amylase activity. Meredith, Eva, and Anderson (5) found that the amylase activities of malted wheat flours prepared from hard red spring wheat malts exhibited marked differences due both to the variety of wheat and points of growth. Kneen, Miller, and Sandstedt (4) applied the techniques for determining alpha- and beta-amylase activity developed in their laboratory to a study of the changes in amylase activity which occurred during the germination of hard red winter wheat at different temperatures in their laboratory. Although these studies were not conducted under malting conditions, they showed that both time and temperature of germination were important factors in the conversion of bound beta-amylase to the free form and in developing alpha-amylase activity.

The present investigation, which was conducted in 1942, represents a continuation of the preliminary study of Geddes, Hildebrand, and

Anderson (2) on the effect of wheat type, environment, and malting conditions on the value of wheat malt for increasing the gas production of doughs in breadmaking. Composite samples of the two highest grades of four market classes of United States' wheats for each of two crop years were each experimentally malted at three moisture levels for five germination times giving a total of 240 samples, and data were obtained for malting loss, cleaning loss, and amylase activity.

Materials and Methods

Composite samples of grades one and two hard red spring, hard red winter, soft red winter, and soft white wheats representing marketings of these wheat classes and grades for the 1940 and 1941 crops, respectively, were malted and the malts studied for amylase activity. The wheat lots represented composites of the official grading samples of the major receipts for these classes at the principal terminal markets.⁴

TABLE I

WHEAT CLASSES, GRADES, AND MOISTURE AND PROTEIN CONTENT OF THE EIGHT COMPOSITE SAMPLES OF THE 1940 AND 1941 CROP

Sample no.	Year grown	Class	Grade	Moisture content %	Protein content ¹ %
1	1940	Soft red winter	2	12.4	10.5
2	1941	Soft red winter	1 and 2	8.6 ²	10.7
3	1940	Hard red winter	2	8.3 ³	13.2
4	1941	Hard red winter	2	9.9	12.7
5	1940	Hard red spring	1	12.2	13.2
6	1941	Hard red spring	1	11.3	12.0
7	1940	Soft white	2	12.3	8.7
8	1941	Soft white	2	10.4	8.0

¹ N \times 5.7; expressed on 14% moisture basis.

² Stored at Wooster, Ohio, until just prior to malting.

³ Stored at Manhattan, Kansas, until just prior to malting.

All other samples stored in unheated granary at Madison, Wisconsin.

The 1940 samples were stored under good conditions until the 1941 samples were received. The two seasons' wheats were cleaned and malted during the spring of 1942. The grade, moisture content, and protein content of the wheats are given in Table I.

Malting. The samples were malted in the Madison, Wisconsin, experimental malting unit at 16°C. (60.8°F.) at three moisture levels for 2, 3, 4, 5, and 6 days germination. The original, cleaned samples were divided through a Boerner divider into 60 approximately 200-g. lots for each class of wheat. Samples of 170 g., dry basis, were weighed out and stored until malted. Duplicate samples were malted as two independent series. The wheat classes for the two seasons were

⁴ Samples were furnished by Robert H. Black, Grain Standards Research, Grain and Seed Division, Agricultural Marketing Service, United States Department of Agriculture.

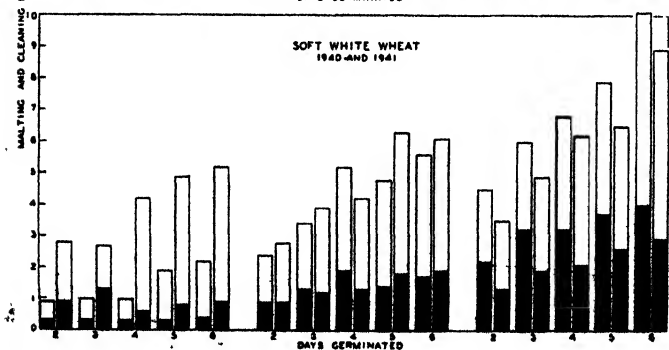
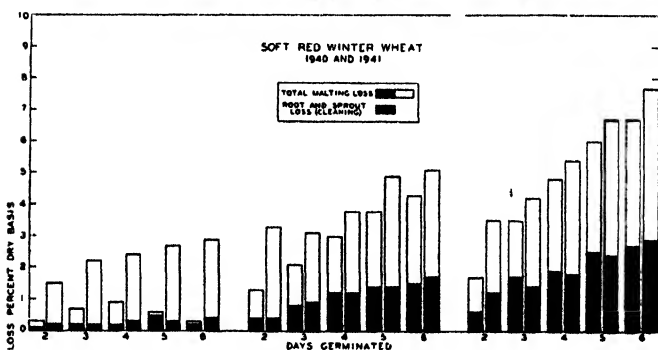
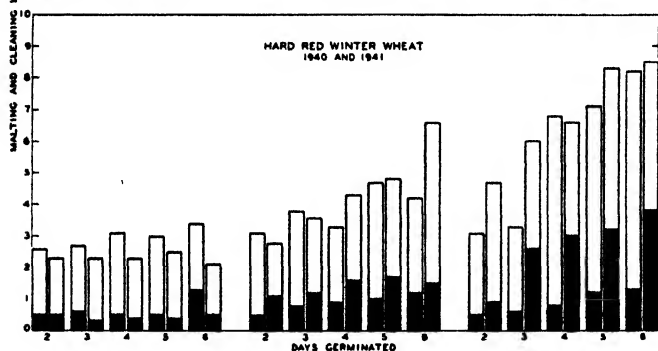
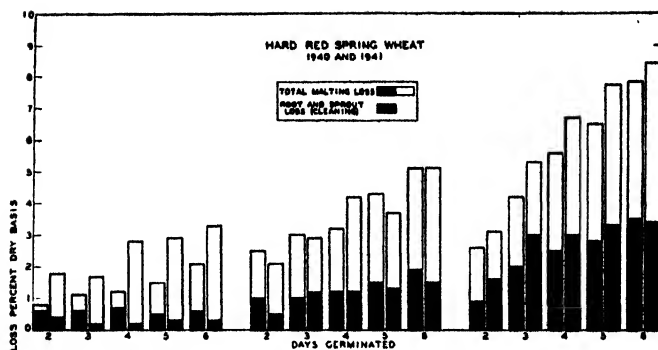
randomized within each duplicate series for each moisture level. The lots of five samples each for 2-6 day germinations were placed in the steep and malting chamber at intervals so that all samples went into the dryer at the same time. The germinated wheats were dried to about 12% moisture according to the following schedule: 8 hours at 25°C. (77°F.), 4 hours at 35°C. (95°F.), 12 hours at 45°C. (113°F.), and 5-7 hours at 55°-75°C. (131°-167°F.). After drying, the samples were weighed, cleaned to remove rootlets and sprouts, reweighed, and stored.

Amylase Activity. Since Geddes and Merritt (3) found that the amylase activity of ground malted wheats and of the flours prepared from them were highly correlated, amylase activity was determined on the malted wheats, thereby eliminating the labor and errors involved in experimental milling. The activity was determined in duplicate on each of the 240 malts, ground in a Wiley mill to pass the 0.5 mm. sieve, by adding increments to a low-diastatic flour measuring the gassing power, and computing the reciprocal of the dosage in per cent necessary to yield the same quantity of gas (205 ml.) as a flour possessing a maltose value of approximately 280 units.⁵ Doughs prepared from 14 g. of flour (including the malt addition) on a 13.5% moisture basis and 3.0% yeast were fermented at 30°C. for 5 hours and the gas production measured volumetrically. The reciprocal of the malt dosage required to give the desired level of gas production was computed from the regression equation for the relation between gas production and the logarithm of the malt dosage (expressed in per cent).

Methods Used in Analysis of Data. The data on the influence of malting conditions on malting loss and amylase activity values of the four wheat classes were evaluated by several methods. Malting the four classes of wheat at three moisture levels and for periods of 2-6 days germination involved some modification of the usual procedures for evaluating data and drawing conclusions. The analysis of variance was used on the data in two ways.⁶ First, an analysis of variance was made on the data using the mean values for the five germination periods; in other words, the effect of germination time was omitted by using the average of the data for the 2-6 day germination periods. Secondly, an analysis was conducted on the logarithmic transformation

⁵ When these studies were undertaken, cereal chemical laboratories commonly evaluated malted wheat flour for breadmaking purposes by determining either the quantities required to give a definite maltose value or dough gas production. It is now generally agreed that the value of malt supplements for breadmaking depends on their alpha-amylase activities. Currently, amylase activities of doughs in excess of those required to maintain adequate sugar levels for the support of yeast fermentation are recommended because of their beneficial effects on bread quality. The favorable influence of amylase supplements on bread characteristics is related to the extent of starch modification by alpha-amylase during oven-baking and measurements of starch degradation during gelatinization have been proposed to replace those heretofore used.

⁶ Credit is gladly given to Dr. C. H. Goulden, Cereal Division, Department of Agriculture, Ottawa, Ontario, Canada, and to Dr. J. H. Torrie, Department of Agronomy, University of Wisconsin, for assistance on this phase of the problem. Dr. Goulden suggested the logarithmic transformation of the data and applied the homogeneity test to verify the validity of the transformation on part of the data.



of the complete data. To avoid negative logarithms, 1.0 was added to all values. Obviously the first analysis gave an incomplete evaluation of the response of the wheat classes as it was impossible by this procedure to show the effect of time on the malting response. The latter method of analysis evaluates the influence of the various factors and the interaction between these factors and wheat classes and years in which the wheats were produced. Applying Bartlett's test (1) for homogeneity to the transformed data gave chi square values which were close to the 50% point so that the differences between the variances were about what would be expected on the basis of random sampling from a homogenous population.

Results and Discussion

The data and mean values for the four classes and two years for malting losses, cleaning losses, and amylase activity values are presented graphically. The data on malting and cleaning loss are presented by means of histograms in Fig. 1. The mean values for each of the three factors are given in Fig. 2. The influence of moisture levels in the steep and during malting and germination time on malting loss and amylase activity values using the mean values for the four wheat classes and two years is shown in Fig. 3. In constructing the graphs the moisture levels were arranged along one axis and germination time along the other. The functions of the moisture reactions at a given time form a series of lines perpendicular to a base plane common to both moisture level and germination time. The relations between moisture and time for the two factors, malting loss and amylase activity values, are shown for the entire moisture and time range. For clarity of presentation, the tops of the vertical lines representing reaction are connected forming a sectional surface or plateau, which

Fig. 1. Malting and cleaning losses for wheat composites grown in 1940 and 1941. The 1940 composites are represented by the histograms on the left of each pair; 1941 composites are on the right. The wheats were steeped at three moisture levels of approximately 35, 40, and 45% reading from left to right.

The actual moisture values were as follows:

Wheat class	Approximate moisture %	Actual moisture	
		1940 %	1941 %
Hard Red Spring	35	33.5	35.2
	40	40.5	40.3
	45	45.5	45.8
Hard Red Winter	35	34.7	34.6
	40	40.0	40.6
	45	46.3	46.0
Soft Red Winter	35	32.3	34.4
	40	39.4	39.9
	45	45.2	45.8
Soft White	35	34.2	34.6
	40	40.5	41.0
	45	46.0	45.5

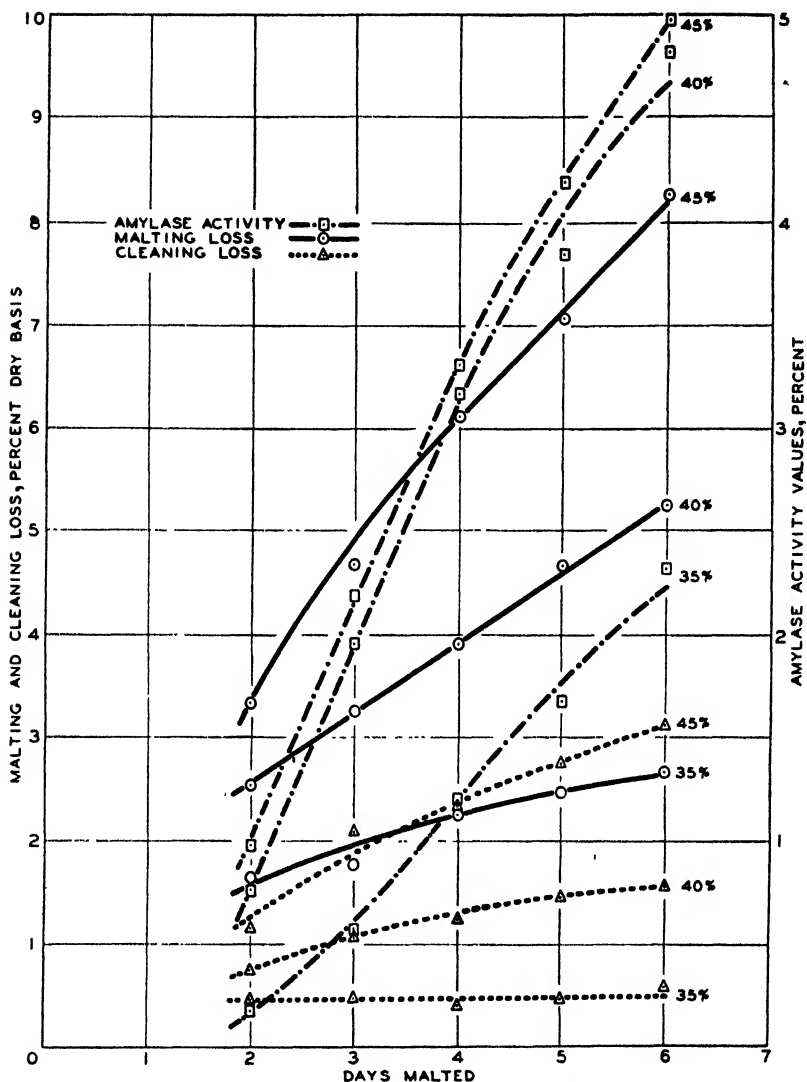


Fig. 2. The influence of moisture and time on malting and cleaning losses and amylase activity in wheat. The curves represent the mean values for the four classes and two years.

represents the interaction between moisture and time. The influence of moisture and time upon the reaction of the factor is shown by the rise in the plateau along the two axes. The reaction of the four wheat classes at the three moisture levels for malting loss and amylase activity values is given in Fig. 4. The construction of the graphs is similar to those in Fig. 3, except that moisture levels and wheat classes are arranged along the two axes. The mean values for 2-6 days germina-

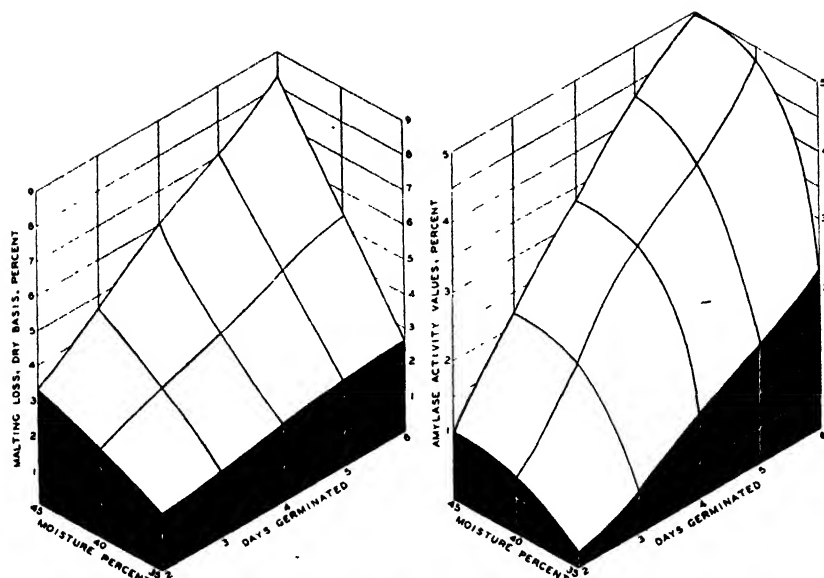


Fig. 3. The influence of moisture levels used in the steep and during malting and germination time in days on malting loss and "amylase activity" values using the mean values for the four wheat classes and the two years. Moisture levels are arranged along one axis and germination time along the other. The functions of the moisture reactions at a given time form a series of lines perpendicular to a base plane common to both moisture level and germination time. The tops of the vertical lines are connected forming a sectional surface or plateau which represents the interaction between moisture and time. The influence of moisture and time upon the factors is shown by the rise in the plateau along the two axes.

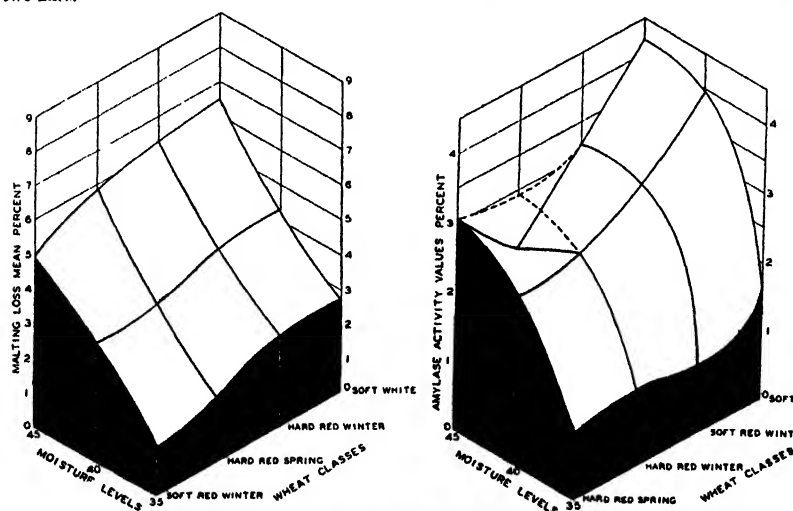


Fig. 4. The reaction of the four wheat classes malted at the three moisture levels for malting loss and amylase activity using the mean values for the five germination times and the two years. The construction of the graphs is similar to those in Fig. 3 except that moisture levels and wheat classes are arranged along the two axes. The sectional surface or plateau represents the interaction between malting moisture and wheat classes. The plateau surface represented by the broken lines is constructed on the basis of no interaction of the hard red winter at the 45% moisture level. The reaction of the wheat classes to malting loss and amylase activity at the three moisture levels is shown by the rise of the plateau along the two axes. The shift in rank of wheat classes for the two factors, malting loss and amylase activity, is shown.

tion periods were used. The sectional surface or plateau represents the interaction between malting moisture and wheat classes. The reaction of the wheat classes to malting loss and amylase activity values at the three moisture levels is shown by the rise of the plateau along the two axes.

Malting Loss. Malting loss, the difference between the original weight, dry basis, of the wheat and the cleaned malt, was used rather than the volume or bushel relationship between wheat and malt. Malting loss included loss in weight due to leaching in the steep water, respiration during steeping and germination, and removal of rootlets and sprouts in cleaning the dried malt. The *F* values obtained by analysis of variance using the logarithmic transformation of the data for malting loss are given in Table II.

TABLE II

ANALYSIS OF VARIANCE OF DATA FOR MALTING LOSS, CLEANING LOSS, AND AMYLASE ACTIVITY VALUES FOR FOUR CLASSES OF WHEAT GROWN IN TWO YEARS MALTED IN DUPLICATE AT THREE MOISTURE LEVELS FOR FIVE GERMINATION TIMES

Source of variation	Degrees of freedom	Mean squares		
		Malting loss	Cleaning loss	Amylase activity values
Wheat classes	3	.198**	.092**	.272**
Years	1	.476**	.040**	.001
Moisture levels	2	2.632**	2.200**	2.019**
Germination time	4	.445**	.189**	1.742**
Wheat X years	3	.078**	.055**	.104**
Wheat X moisture levels	6	.019	.031**	.043**
Wheat X germ. time	12	.005	.005	.006
Years X moisture levels	2	.105**	.030**	.015
Years X germ. time	4	.002	.001	.009
Moisture levels X germ. time	8	.026	.042**	.018
Error	120	.016	.005	.012

Based on the two years' samples, malting losses were influenced significantly by wheat class, year, moisture, and time of malting. The wheat classes ranked in ascending order for the mean malting loss of all moisture levels and germination periods for each year were: soft red winter, hard red spring, hard red winter, and soft white wheat.

The malting losses were higher generally for the 1941 wheats than for those collected in 1940. The wheat classes varied in malting loss within the two years sampled and they responded differently when malted at different moisture levels (Table II). While there was considerable variation between duplicates, the upward trends for the increases due to longer germination times and higher moistures were

highly significant. The curves for comparative mean malting loss for the four classes and two years combined, Figs. 2 and 3, indicate the relationships to moisture and time.

Cleaning Loss. In cleaning the dried wheat malts, the rootlets and many of the sprouts were removed. The cleaning loss represented the weight of rootlets and sprouts removed and was a rough, comparative measure of growth. Cleaning loss was included in the malting loss discussed previously (Figs. 1 and 2 and Table II).

Cleaning loss was influenced by wheat classes, years, moisture levels, and germination time, as in the case of malting loss. Significant interactions for wheats \times years and years \times moisture levels were again similar to malting loss. In addition, significant interactions for wheats \times moisture levels and moisture levels \times germination time occurred in cleaning loss. In the hard red winter class for 1940 the cleaning loss for samples malted at 45% moisture was lower than for those malted at 40% moisture. The increase in cleaning loss with increased germination time varied considerably, especially at the 35% moisture level where moisture was too low for good root and sprout growth.

Amylase Activity. The amylase activities of the malts were influenced significantly by wheat class and especially by the moisture and time of malting. Significant interactions were found for wheats \times years and for wheats \times moisture levels. For the two years and over-all malting conditions, soft white wheat yielded malt of the highest activity followed by soft red winter, hard red winter, and hard red spring wheat in the order named. The interaction of years by wheat classes is due largely to a change in the relative ranking of hard red spring and hard red winter wheats. For 1940 the activities of their malts fell in third and fourth place, respectively, while the reverse was the case for the 1941 samples. With an increase in the moisture content at which the samples were germinated, there was a greater increase in the amylase activity of the malts prepared from soft white and soft red winter wheats than of those from the hard red spring and hard red winter wheats.

The contrast between the shape of the plateaus (Figs. 3 and 4) for malting loss and amylase activity values suggests a linear relation in the former and a quadratic relation in the latter. Tests for linear and quadratic functions of the two sets of data show this to be the case.

Simple correlations were computed between the data on malting loss and amylase activity values. The positive correlation ($r = +0.710$) between the two factors was highly significant, indicating that in these wheat classes for the two years sampled, malting loss might be used as an index of the relative amylase activity to be

expected. In practical application malting loss can be evaluated against amylase activity.

The relative influence of moisture level on malting loss and amylase activity values, Fig. 4, suggests the use of moisture levels around 40% as the most economical for wheat malting. The increased loss in dry weight in malting at 45% moisture in contrast with the loss at 40% was not compensated by a proportional increase in amylase activity. This relationship was indicated for all four classes of wheat.

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EFFECT OF WHEAT CLASS AND MALTING CONDITIONS ON GASSING-POWER STIMULATION AND ALPHA- AND BETA-AMYLASE ACTIVITIES OF MALTED WHEAT¹

W. F. GEDDES,² J. G. DICKSON,³ and C. B. CROSTON⁴

ABSTRACT

Nineteen composite samples of wheat malts representing four wheat classes and variations in germination moisture and time were analyzed for free and total alpha- and beta-amylase activities. The activities, expressed as a reciprocal of the percentage addition of each malt required to produce a given stimulation in the gas production of a fermenting dough, were also ascertained. The free and total alpha-amylase values agreed closely and increased in parallel fashion with increases in germination moisture and time. In contrast, the total beta-amylase was virtually constant for all malts, but as germination progressed, there was a marked increase in free beta-amylase. The various positive correlations between the different measures of amylase activity were all of a very high order of magnitude.

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The partial correlation between free alpha-amylase activity and the reciprocal of the malt dosage required to give a uniform gas stimulation, independent of free beta-amylase activity, was $+0.92$, in contrast to a low and insignificant correlation of $+0.14$ between beta-amylase activity and reciprocal malt dosage, independent of free alpha-amylase activity. These results support the widely held view that the alpha-amylase activity of malts is the important factor in relation to their stimulating effect on the gas production of fermenting doughs.

The wheat malts prepared by Dickson and Geddes (1) in their investigation of the effect of wheat class and malting conditions on the value of the malts for increasing the gas production of wheat flour doughs provided valuable material for a study of the development of free and total alpha- and beta-amylase activities at various stages of the malting process and the relation of these activities to gas stimulation in doughs. Since Kneen, Miller, and Sandstedt (2) had just reported a comprehensive investigation of the changes in amylase activity that occurred during the germination of hard red winter wheat at various temperatures, the present study was limited to a series of 19 composites representing the different wheat classes, germination moistures, and germination times.

Materials and Methods

Nineteen composites were prepared from equal weights of certain of the individual ground malts described by Dickson and Geddes (1). One composite was prepared for each of the three germination moistures (approximately 35, 40, and 45%) and for each of five germination times (2 to 6 days inclusive) to give a total of 15 composite samples, each of which contained 16 individual malts representing duplicate maltings for each of two crop years of hard red spring, hard red winter, soft red, and soft white wheats. Another set of four composites was prepared which represented the four respective wheat classes; in this series, each composite contained 24 individual malts, which represented duplicate maltings for the two crop years at two moisture levels (approximately 40 and 45%) and three germination times (4, 5, and 6 days).

The techniques used by Kneen, Miller, and Sandstedt (2) in a similar study were followed, with certain minor modifications. Preliminary experiments showed that the dextrinogenic activities of extracts made with 0.1 and 0.2% calcium acetate solution were very similar and somewhat higher than corresponding extracts made with distilled water. As 0.1% calcium acetate extracts gave beta-amylase values in close agreement with those for distilled water extracts, there was no evidence of an inactivating effect (5) of calcium ions at this concentration; accordingly, both the free and total extracts were made

by extracting 1.0 g. of malt with 100 ml. of 0.1% calcium acetate solution. This ratio of malt to liquid was used to avoid subsequent dilution, or the use of less than 1.0 ml. of the more highly active extracts, in the dextrinogenic determinations. A two-hour extraction time at 30°C. was used in preparing the free extract, whereas the total extract was obtained by employing 0.5 g. of papain (Parke, Davis and Company) per gram of malt with an extraction time of 18 hours at 30°C. Preliminary trials showed that this quantity of papain was required to ensure maximum total saccharogenic activity.

The alpha- and beta-amylase activities of the malt extracts were determined by the respective methods of Sandstedt, Kneen, and Blish (7) and of Kneen and Sandstedt (3).

Results and Discussion

The results are summarized in Table I together with the corresponding mean amylase activities determined by ascertaining the reciprocal dosage of malt required to produce a given stimulation in the gas production of a fermenting dough. These mean amylase units were computed from the appropriate data obtained by Dickson and Geddes (1) for the individual malts. The correlation coefficients involving these different measures are given at the foot of the table.

The data for these malts which were prepared under conditions similar to those used in commercial malting confirm the general trends noted by Kneen *et al.* (2) who used a "rag doll" technique for malting. The free and total alpha-amylase values are in close agreement and increase in parallel fashion with increases in germination moisture and time. In contrast, the total beta-amylase is virtually constant for all the malts, but as germination progresses there is a marked increase in free beta-amylase. However, only approximately 50% of the total beta-amylase was present in the free condition even after germinating wheat containing 45% moisture for six days (at 16°C.). This is not in agreement with the results obtained by Kneen *et al.* who found that the free and total activities became essentially equal during the later stages of germination, nor is it in accord with the experience with barley malts. Wheat class appears to have some influence on the percentage of the total beta-amylase which is present in the free state under corresponding malting conditions, but one would hardly expect the class or variety of wheat to be the complete explanation for these differences. Variations in the severity of the heat treatment employed in drying the malts in different studies would influence their amylase activities.

The various correlations between free alpha-amylase, total alpha-amylase, free beta-amylase, and amylase activity determined by the

gas production technique are all of a high order of magnitude. The correlation of +0.984 between free (as well as total) alpha-amylase activity and the activity measured by the gas stimulation method

TABLE I
EFFECT OF MALTING CONDITIONS AND WHEAT CLASS UPON AMYLASE ACTIVITIES

Nature of sample		Alpha amylase ¹			Beta-amylase ²			Amylase activity from gas production ³
Germination conditions								
Approx. moisture %	Time days	Free	Total	Free as % total	Free	Total	Free as % total	1/g.
Based on Composite Malts from Four Wheat Classes ⁴								
35	2	3.8	4.6	83	4.5	19.6	23	0.10
	3	9.3	10.5	88	4.9	19.7	25	0.27
	4	17.9	21.2	84	5.4	20.3	27	0.57
	5	28.8	30.8	93	6.6	20.4	32	0.80
	6	43.8	42.0	104	7.6	20.6	37	1.11
40	2	13.6	15.3	89	4.6	20.1	23	0.38
	3	36.6	36.7	100	6.0	20.6	29	0.99
	4	58.6	58.6	100	7.8	20.5	38	1.58
	5	68.5	71.9	95	9.1	20.5	44	2.24
	6	89.6	91.0	98	10.1	20.5	49	2.41
45	2	19.0	19.0	100	6.7	19.8	34	0.50
	3	39.8	39.6	100	8.3	20.2	41	1.09
	4	61.4	65.2	94	9.5	20.4	47	1.65
	5	76.1	75.3	101	10.2	20.4	50	2.15
	6	91.4	96.1	95	10.3	21.0	49	2.48

Based on Composite Malts Germinated at Approximately 40 and 45% Moisture for 4, 5, and 6 days

Wheat class	Free	Total	Free as % total	Free	Total	Free as % total	Amylase activity from gas production ³
Soft white	77.3	79.6	97	9.6	15.2	63	2.65
Soft red winter	71.9	74.4	97	12.8	22.1	58	2.22
Hard red spring	64.8	71.3	91	10.1	22.4	45	1.83
Hard red winter	56.8	62.9	90	8.2	19.2	43	1.64

Correlation Coefficients

Free alpha-amylase \times amylase activity (gas production) = +0.984
 Total alpha-amylase \times amylase activity (gas production) = +0.984
 Free beta-amylase \times amylase activity (gas production) = +0.902
 Free alpha-amylase \times total alpha-amylase = +0.997
 Free alpha-amylase \times free beta-amylase = +0.905
 Total alpha-amylase \times free beta-amylase = +0.904
 (Value of r at the 1% point = 0.575.)

¹ The alpha-amylase unit is the number of grams of soluble starch which is dextrinized by the alpha-amylase of 1.0 g. malt (dry basis) in 1.0 hour at 30°C. in the presence of excess beta-amylase.

² The beta-amylase unit is the number of grams of soluble starch converted to maltose by the beta-amylase of 1.0 g. malt (dry basis) in 1.0 hour at 30°C.

³ Reciprocal dosage of malt required to produce a given stimulation in gas production.

⁴ The beta-amylase activity of a composite sample of the four wheat classes was: free beta-amylase = 3.8 units; total beta-amylase = 20.0 units; free as % of total = 19%.

indicates that the increased gassing power of a dough upon the addition of malted wheat meal is almost entirely dependent on alpha-amylase activity. This supports the conclusion of Kneen and Sandstedt (4) that determinations of alpha-amylase and increased gassing power "appear to be equally reliable for evaluating malts." These workers obtained a correlation of 0.94 between these two measures. On the other hand, Meredith *et al.* (6), employing malt flours milled from 39 malted wheats prepared from composite samples of wheat representing several hard red spring wheat varieties and a number of locations in western Canada, obtained correlations of 0.71 for varieties and 0.79 for stations. They point out that a close association between two such properties in a series of samples which are produced by progressively changing the processing methods does not necessarily indicate a causal relation.

In the present study free beta-amylase and amylase activity by the gas production method were highly correlated (+0.902); as the correlation between free alpha- and free beta-amylase is also quite high, the simple correlation coefficients do not give a measure of the relative importance of alpha- and beta-amylase in relation to the stimulation of gas production in doughs. The partial correlation between free alpha-amylase activity and the reciprocal of the dosages of malt required to give a uniform gas stimulation, independent of free beta-amylase activity, is +0.916, whereas the corresponding correlation of +0.144 between beta-amylase activity and reciprocal malt dosage, independent of free alpha-amylase activity, is not statistically significant. These results, therefore, support the widely held view that the alpha-amylase activity of malts is the important factor in the stimulation of gas production which is obtained when malt products are added to fermenting doughs.

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COMMUNICATION TO THE EDITOR

A Method for Staining Insect Egg Plugs in Wheat

DEAR SIR:

With the attention now being given to mill sanitation, it is becoming increasingly necessary that a more satisfactory method be developed for the identification of weevil infestation in wheat. The use of stains, such as acid fuchsin and iodine-potassium iodide, has been reported, but they have the disadvantage of staining exposed endosperm as well as weevil egg plugs. The gentian violet procedure given below is a selective stain for insect egg plugs and, under the conditions described, will not stain the endosperm of the wheat kernel.

The wheat sample (usually 5 g.) is steeped in warm water containing a wetting agent. Three drops of Triton W-30 (a sulfated aromatic ether alcohol; Rohm and Haas Co., Inc.) in 200 ml. of water will satisfactorily wet the wheat in approximately 30 seconds. Other wetting agents, such as Aerosol OT (dioctyl sodium sulfosuccinate; American Cyanamid Co.) and Sterox SK (a polyoxyethylene thioether; Monsanto Chemical Co.), also are satisfactory. The sample is easily handled if it is placed in a small wire container or in a container with a wire bottom so that it can be transferred from one solution to another. When the sample is removed from the wetting solution, the excess water should be removed by placing the container with the wet wheat, or the wet wheat alone, on a dry towel for a few seconds before putting the sample into the staining solution.

The wheat is exposed for 2 minutes in a solution which contains 10 drops of a 1% aqueous stock solution of gentian violet (Coleman-Bell Co.) in 50 ml. of 95% ethanol. The sample then is removed from the staining reagent and washed in clear water for approximately 20 seconds, or until the excess stain held by the brush end of the kernel is removed. The insect egg plugs are a purple color and are very easily distinguished at 5 to 7 magnifications. The egg plugs are seen most clearly while the kernels are wet or if the sample is placed in water.

Good results are obtained by this gentian violet staining technique, but there are some precautions that should be observed. The insect egg plugs in wheat at the usual moisture content will not stain in an alcoholic solution unless the kernels are first wet with water. On the other hand, if the excess water is not removed from the wetted sample, the wheat kernel will stain completely. Also, if the wheat is badly

damaged and the bran is torn or cracked, one of the under layers of the pericarp will stain around the edge of the tear. This, however, will not be confused with the insect egg plugs nor will it obscure or prevent any egg plugs from staining.

Although the aqueous stock solution has a long storage life, the efficiency of the dilute alcoholic stain becomes unsatisfactory when the solution takes on enough water to stain or tint the entire wheat kernel. The number of samples that can be stained in one solution depends upon the wetness of the wheat placed in this alcoholic solution.

This gentian violet staining procedure, when the above-mentioned precautions are observed, gives more satisfactory results than other procedures.

July 6, 1949

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BOOK REVIEWS

Advances in Food Research. Volume I. Edited by E. M. Mrak and George F. Stewart. 459 pp. Academic Press Inc., New York, N. Y. 1948. Price \$7.50.

The book contains 10 chapters dealing with various phases of Food Chemistry. Bate-Smith from Cambridge, England, gives an excellent review of the present status of knowledge concerning the physiology and chemistry of rigor mortis with special reference to the aging of beef. This article should be of special interest to chemists in the meat packing industry since it relates the changes occurring during rigor mortis to the glycogen content, the pH, the color of the flesh, the rate of penetration of salt during curing, the amount of drip from frozen meats after thawing, and the effects these factors have on the increase in tenderness during the ripening process.

Clifcorn of the Continental Can Company, Chicago, gives a detailed review of the factors influencing the vitamin content of canned foods.* This review is of particular interest to people in the canning industry and in the field of nutrition. In various tables he gives the vitamin content of a variety of different kinds of canned foods and shows how these may be affected by the canning operations, by storage, and by the processes used preparatory to the serving of the foods.

Lepovsky from the University of California at Berkeley has a very interesting chapter on the physiological basis of voluntary food intake. He discusses such questions as whether or not humans and animals can choose foods wisely in accordance with their nutritional needs, the effect of proteins, amino acids, water-soluble vitamins, fats, and fat-soluble vitamins and minerals on the voluntary food intake. The factors influencing voluntary food intake are governed by very complex physiological mechanisms that are not too well understood, but the author gives a very good review of our present state of knowledge.

Lightbody and Fevold of the Quartermaster Food and Container Institute, Chicago, give a detailed discussion of the biochemical factors influencing the shelf life of dried whole eggs and means for their control. This article should be of extreme importance to those chemists who are concerned with the preparation of dried egg products. A great deal of research was carried out during the recent war on the factors responsible for the deterioration in quality of dried egg products during drying and storage. The authors give an excellent review of this research and bring out the factors that must be controlled if a good quality powder is to be produced and one that will retain its qualities during storage.

Miss Lowe of Iowa State College, Ames, Iowa, discusses the factors affecting the palatability of poultry products. She discusses the various factors that can influence quality with particular reference to those which can be controlled during processing.

Some excellent photomicrographs are included that show the structure of the protein and the changes that take place in the structure during aging.

Ross, Cornell University, Ithaca, New York, has an excellent review on the deterioration of processed potatoes. The article deals primarily with the problems involved in the production of dehydrated potatoes and shows what types of deterioration can take place in this product. The browning reaction receives the bulk of attention and the author discusses in detail various factors that contribute to browning and how it can be controlled.

Somers and Beeson discuss the influence of climate and fertilizer practices upon the vitamin and mineral content of vegetables.

Stadtman, University of California, Berkeley, has a comprehensive article on the nonenzymatic browning in fruit products. This is an excellent review of the theories and the effects of the various environmental factors on the browning reaction. After reading this article one appreciates the fact that the browning reaction is indeed complex and that a thorough understanding is necessary for proper control.

Wyss of the University of Texas, Austin, Texas, has a short review on the microbial inhibition by food preservatives. This article is not a catalogue of the microbial inhibitors that are used in foods but rather an academic treatment of the topic.

Baker, University of Delaware, Newark, discusses high-polymer pectins and their deesterification. This is a concise treatment of the topic, including the composition, source of materials, extraction methods, and methods of deesterification. He also indicates the properties and uses of the low ester pectins.

This is the first volume of *Advances in Food Research*. This volume should be an extremely valuable book for all food technologists, and if future volumes in this series continue in the same high level of quality as exhibited in the first volume, this series will make valuable reference books in the library of all food technologists. Dr. Mrak and Dr. Stewart are to be congratulated on a job well done.

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Sugar. By Andrew Van Hook. 155 pp. illus. Ronald Press Company, New York. 1949. Price \$3.00.

This book should meet the needs of the reader interested in the nature and production of sugar (sucrose). Technical details necessary to an understanding of the formation of sugar in the plant, manufacture of the finished sugar, the place of sugar in world trade, and the uses of by-products have been well presented so that the average reader gains a good understanding of all phases of sugar production.

The book is made up of six chapters. Chapter I, an introduction, deals with the structure of matter, chemistry, particularly as related to sugar, and finally the uses of sugar. This is the least interesting chapter of the book, but doubtless will be useful to some readers.

Chapter II discusses the production of sugar cane and the cane sugar refined from it, with greatest emphasis upon factory processing. The various steps in factory processing have been dealt with in a brief but clear fashion. The reader should have no difficulty in grasping the fundamentals if not the details of cane sugar processing.

Chapter III deals with the production of sugar beets and beet sugar in much the same manner that the previous chapter deals with cane. Again the discussion is brief, but adequate.

Chapter IV, Commercial and Trade Aspects, discusses world production and consumption of sugar, as well as production by countries. The statistical data presented appear to be accurate and up to date.

Chapter V takes up the problem of by-products, cane bagasse, beet pulp, molasses, and possible uses of sugar in the production of chemicals. Use of sugar beet tops, a valuable farm by-product, is not discussed.

Chapter VI, History, should be of considerable interest to the reader who is interested in the growth and development of the sugar industry to its present status.

The book is well illustrated and indexed and the format is excellent.

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SUGGESTIONS TO AUTHORS

General. Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, all original drawings or photographs for figures, and one set of small photographic reproductions of figures.

Issues of *Cereal Chemistry* published after January 1, 1948, when some changes were made, are the most useful guides to acceptable arrangements and styling of papers.

Titles and Footnotes. Titles should be specific, but should be kept short by deleting unnecessary words. The institution from which the paper is submitted, author's connections, etc., are given in footnotes.

Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. Long introductory reviews should be avoided, especially when a recent review in a monograph or another paper can be cited instead. References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also.

Organization. The standard organization involves main sections for abstract, introduction, materials, methods, results, discussion, acknowledgments, and literature cited. Alternately, a series of separate studies is often best described with main sections for each study. Headings should be restricted to center headings for main sections and run-in italicized headings for subsections.

Tables. Data should be condensed and arranged to facilitate the comparisons the reader must make. Tables should be kept reasonably small by breaking up large ones, omitting all unessential data, and minimizing number of significant figures. Leader tables without number, main heading, or ruled lines are useful for small groups of data. Textual matter in tables should be minimized and unnecessary footnotes should be avoided.

Tables should be typed on separate pages at the end of the manuscript and their positions should be indicated to the printer by typing "(Table I)" in the appropriate place between lines of the text. (Figures are treated similarly.)

Figures. If possible, all drawings should be made by a competent draughtsman. Curves should be drawn heaviest, axes or frame intermediate, and grid lines lightest. The horizontal axis should be used for the independent variable; and experimental points should be shown. Labels are preferable to legends. All drawings should be made two to three times eventual reduced size with India ink on white paper, tracing linen, or blue-lined graph paper. Lettering should be done with a guide, and letters should be $\frac{1}{8}$ to $\frac{1}{4}$ inch high after reduction.

For difficult photographs, a professional should be hired or aid obtained from a good amateur. The subject should be lighted to show details. A bright print with considerable contrast reproduces best, and all prints should be made on glossy paper.

Figures should be identified by lightly writing number, author, and title on the back. Cut-lines (captions) should be typed on a separate sheet attached to the end of the manuscript.

Style. Clarity and conciseness are the prime essentials of a good scientific style. A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*.

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WHEAT PROTEIN AND THE BIURET REACTION¹

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ABSTRACT

The biuret reaction has been applied to the quantitative estimation of protein in wheat and wheat flour. The procedure is simple, rapid, and inexpensive. The protein, peptized by 0.05 *N* potassium hydroxide solution, is treated with copper sulfate stabilized in alkaline solution by a small amount of glycerol. Color intensity of the reddish-violet product, which is proportional to the protein concentration, is determined with a colorimeter. Approximately one-tenth of the protein remains unpeptized and, therefore, not directly measured. Nevertheless, biuret color values are closely correlated with crude protein values as determined by the Kjeldahl method. It is well known that bread loaf volumes are generally closely correlated with Kjeldahl protein content. In this study, they are shown to be correlated about equally well with biuret color values.

In applying the method, biuret values may be evaluated in terms of either total protein or peptized protein, as desired, by means of a graph, table, or formula derived from the biuret and protein values of suitable test samples.

Protein content of wheat has long been recognized as a useful index of its value for breadmaking purposes. The Kjeldahl method for determining protein is accepted as standard, but certain disadvantages of the method are well known. One of the objectives of the Production and Marketing Administration and of the Bureau of Plant Industry, Soils, and Agricultural Engineering has been the development of a test that is simpler, faster, and less expensive than the Kjeldahl method and at least equally as useful for evaluating wheat. In the search for such a substitute, attention was directed to the biuret reaction, which has long been used as a qualitative test and more recently as a quantitative test for protein in biological materials.

When copper in strongly alkaline solution reacts with protein material, a reddish-violet substance is formed. Under proper condi-

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This study was conducted jointly by the Bureau of Plant Industry, Soils, and Agricultural Engineering and the Grain Branch, Production and Marketing Administration, both of the United States Department of Agriculture.

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tions, the intensity of the color produced is proportional to the protein concentration. Copper may react in a similar manner with non-protein materials which contain the amino and carboxyl groups characteristic of proteins.

Wiedemann (1) has been credited with first observing the reaction with biuret, $\text{NH}_2\text{CO. NH. CONH}_2$. Ritthausen and Pott (8) discovered in 1873 that proteins also react in this way and shortly thereafter the reaction came into common use and was called the "biuret" test.

The chemistry of the reaction was studied thoroughly in 1896 by Schiff (10). Riegler (7) in 1914 and Autenreith and Mink (2) in 1915 adapted the test to the quantitative estimation of protein in biological fluids.

Riegler used egg albumen in preparing a "comparison liquid" while Autenreith and Mink used purified biuret as a standard. Little use was made of the new method for several years. In 1928 Hiller (5) made some minor changes in the method and confirmed its usefulness. In 1934 Fine (4) made some further refinements, but his chief contribution was the use of blood serum as a standard.

Robinson and Hogden (9) in 1940 studied and summarized the method and have supplied a good review of the literature.

Materials and Methods

In the biuret test as it has usually been employed, copper sulfate and protein are brought together in a solution containing 3% or more of sodium hydroxide. In the presence of alkali in this concentration, most of the copper is quickly precipitated as the hydroxide, but a small amount is maintained in solution as a complex ion. This is essential to the completion of the reaction which is relatively slow, probably because it involves the complex protein molecule.

The peptization of wheat protein is apparently more nearly complete in 0.05 *N* alkali than in alkali of any other concentration. Accordingly, this concentration was used in our first attempts to apply the biuret reaction to wheat protein. A fair degree of correlation with Kjeldahl protein values was noted, but results obtained were so variable as to indicate the probability that in successive tests the reaction was stopped in varying stages of completion by premature removal of copper by precipitation. This difficulty was overcome by the application of Mehl's (6) proposal in 1945 that the precipitation of copper be avoided by the introduction of ethylene glycol. Glycerol, proposed for this purpose by Sols (11) in 1947, proved even more effective as much smaller quantities of it are required.

The following equipment, reagents, and procedure were used throughout this study:

Equipment

1. Balance, analytical.

Samples were weighed with about the same degree of precision as is ordinarily used in the Kjeldahl procedure.

2. Shaker—motor-driven rack which inverts stoppered bottles about 60 times a minute.

3. Centrifuge—Clay Adams "Senior." Speed 4000 r.p.m.

4. Mill—"Labconco."

5. Colorimeter—Klett-Summerson (12) photoelectric. Fitted for 1 cm. cuvettes, parallel face; No. 54 K-S light filter.

Reagents

1. Stock solutions

- (a) Potassium hydroxide

Prepare a saturated solution, let settle until clear. Dilute the saturated solution to exactly 10.0 *N*.

- (b) Sodium hydroxide

Prepare a saturated solution, let settle until clear. Dilute to 6%; 100 ml. contains 6 g. of NaOH.

- (c) Glycerol—2% solution

100 ml. contains 2 g. of glycerol.

- (d) Copper sulfate

Select only pure crystals of the pentahydrate. Make 4% solution; 100 ml. contains 4 g. of $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$.

2. For use

- (a) Potassium hydroxide

Dilute 50 ml. of 10 *N*. to 10 liters—dilute solution is approximately 0.05 *N*.

- (b) Biuret reagent

Mix thoroughly 60 ml. of 6% sodium hydroxide solution, 160 ml. of 2% glycerol solution, and 740 ml. of distilled water. While stirring vigorously, pipette in slowly 40 ml. of 4% copper sulfate solution. The resulting solution should be clear, deep blue, and stable for several weeks. It should not be used after any precipitate forms and should be stored in an alkali-resistant glass bottle.

Procedure

Mix 2 g. of flour or freshly and finely ground wheat with about 5 ml. of carbon tetrachloride in a bottle. Pipette into the bottle 50 ml. of 0.05 *N*. potassium hydroxide solution and shake for 10 minutes

in a shaker, or by hand if no shaker is available. Centrifuge the protein dispersion or a portion of it until very nearly clear. With accurate pipettes, measure 5 ml. of the clear centrifugate and 15 ml. of the biuret reagent into a test tube. Mix by inverting 3 or 4 times. Let stand for 1 hour or longer, then determine the intensity of color of the reddish-violet solution with the colorimeter. Convert colorimeter scale readings to protein values by means of a chart, table, or formula. (This will be discussed later.)

Discussion

Results obtained by the Kjeldahl method may differ from those obtained by the biuret method as here proposed. In the Kjeldahl procedure, total nitrogen is determined. From this the protein content is calculated with no distinction between protein and nonprotein nitrogen. The biuret reaction involves the peptide linkage and, therefore, ordinarily may be expected to furnish a fairly accurate measure of true protein. As the reaction is here applied, the entire amount of the protein in the sample tested is not directly determined since the peptizing agent leaves unpeptized a small fairly constant fraction of the wheat or flour protein. This portion appears to include chiefly nonendosperm and, therefore, nongluten proteins.

The colorimeter does not distinguish between color intensity and turbidity. If color intensity is to be accurately measured, the colored solution should be as nearly clear as possible. If the lipids present in the wheat and flour are not removed before the protein is peptized, a slight variable turbidity is produced. This turbidity is reduced by removing a major portion of the lipids with carbon tetrachloride, which is in turn eliminated by the centrifugation.

The biuret reagent as here used contains sufficient copper to react with the protein in samples of wheat or flour in which the protein content is not greater than 18%. If it is necessary to test samples in which the protein content is greater than this value, more copper might be included in the reagent, more of the reagent might be used, or smaller samples might be taken. If any of these changes is made, it becomes necessary to prepare a new chart or table for determining the protein values.

The biuret reaction apparently requires about 20 to 40 hours to reach completion. It proceeds rapidly for the first 5 to 10 minutes, then more slowly until at 30 to 60 minutes it is very nearly complete. The change in color intensity is so slow as to be nearly imperceptible during the next 3 hours. Changes of 2 scale units have been noted over a period of 20 hours after the first hour.

Robinson and Hogden (9), Mehl (6), and others have shown that optical densities of biuret test solutions have a maximum value at approximately $550\text{ m}\mu$. This is found to be true also when glycerol is used in the test. This may be seen in Fig. 1. When copper is not permitted to precipitate, as in the presence of glycerol or ethylene glycol, part of the color in the test solution comes from the copper which has not reacted with protein. This part is *zero* if the quantity of protein present is sufficient to combine with all of the copper, and is greatest if *no* protein is present, as in a "blank" test. The color re-

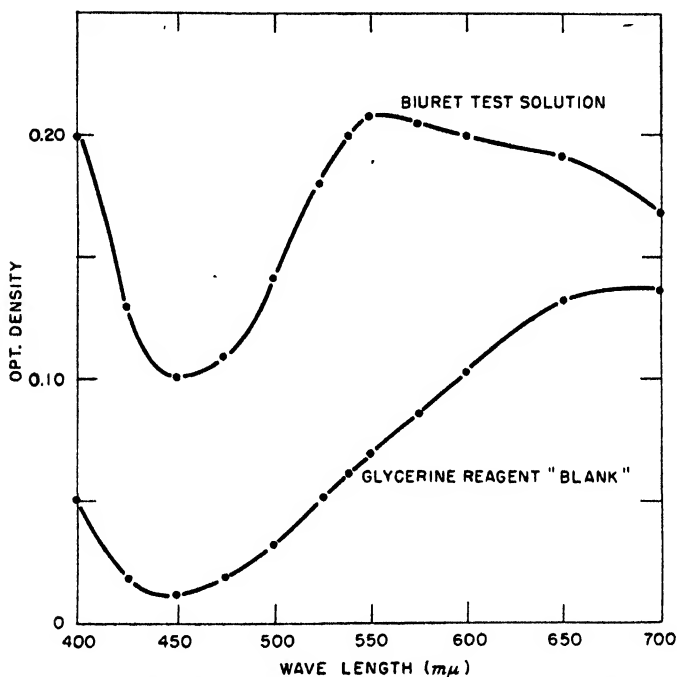


Fig. 1. Optical densities of biuret test solutions at different wave lengths.

sponse of such a test is also shown in Fig. 1. It may be noted that the greatest difference in optical density between "blank" and "biuret" occurs at about $550\text{ m}\mu$. The light filter "K-S No. 54" has a spectral range of approximately 500 to $570\text{ m}\mu$, centered at $540\text{ m}\mu$, and is appropriate for this work. Data for Fig. 1 were obtained by use of a Coleman Junior Spectrophotometer, zero adjustment made with water.

Earlier workers agree that the concentration of sodium hydroxide in the test solution should be no less than 3%. Preliminary experiments in our laboratory indicated that the much lower concentration

of 0.3% is equally satisfactory if ethylene glycol or glycerol is present to stabilize the copper. In Table I are shown the results of an experiment designed to test this observation. Twenty-three samples of wheat were tested as described above. The biuret values of these are shown in column "A." From each of the clear centrifugates prepared for the test a second 5 ml. aliquot was drawn. To it was added 15 ml. of a modified reagent which differed from the usual glycerol-copper

TABLE I
EFFECT OF ALKALI CONCENTRATION IN BIURET TEST SOLUTIONS

"A" NaOH 0.3% K-S scale	"B" NaOH 3.0% K-S scale	Difference	Protein % (Kjeldahl)
132	134	2	15.0
135	135	0	14.2
125	127	2	12.9
137	137	0	15.0
123	125	2	13.1
119	122	3	12.8
121	123	2	13.0
103	109	6	9.9
110	113	3	11.0
99	102	3	10.0
106	109	3	10.4
138	139	1	14.4
115	117	2	12.4
123	125	2	13.2
107	109	2	11.2
106	108	2	11.3
121	122	1	12.6
123	123	0	13.1
140	140	0	15.0
133	132	-1	14.0
130	130	0	13.6
121	122	1	12.5
118	119	1	12.4

Average
difference 1.6

Correlation, biuret values, and protein

	"A"	"B"
r	0.980	0.972
S _{y.x}	0.30	0.35

reagent only in the content of sodium hydroxide, which was sufficient to make the final concentration 3%. Biuret values thus obtained are shown in column "B." Each set of biuret values has been correlated with the Kjeldahl protein values shown in the third column. It is evident that either reagent will produce satisfactory results.

Material. In connection with another project, several hundred samples, each representing a carload lot of commercial wheat, were taken at the larger grain terminals. From these were selected 100

samples of hard red winter wheat, 36 samples of hard spring wheat, and 28 samples of hard white wheat, representative of the wheat received in 11 markets. The usual range of protein content and the grades of wheat are fairly well covered. Each sample of hard red winter

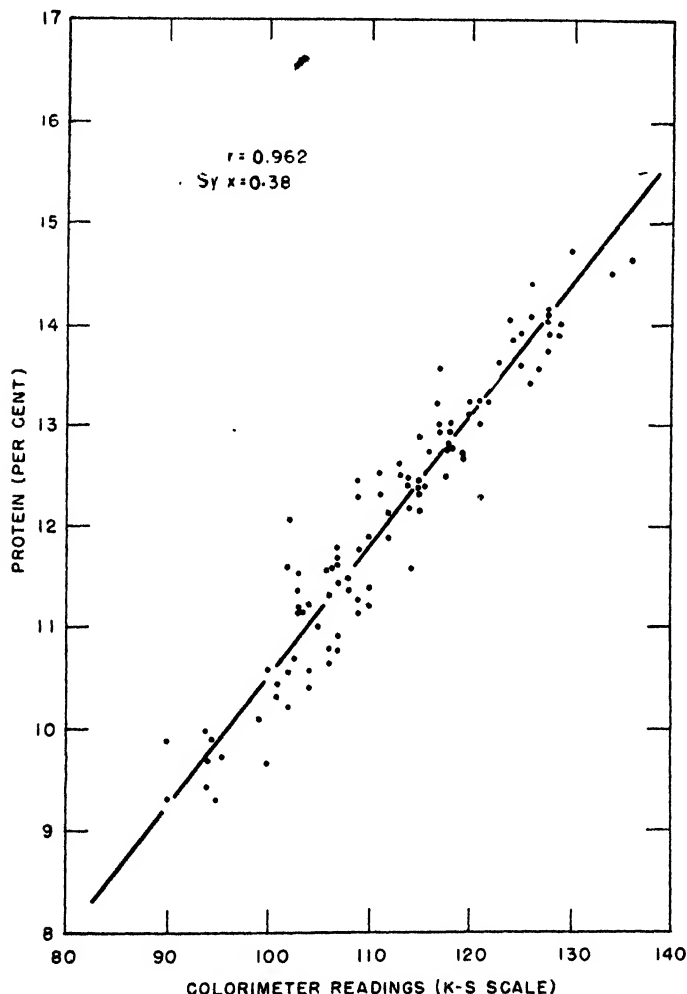


Fig. 2. Correlation of bluret and Kjeldahl protein values of hard red winter wheat.

wheat was milled to 90% patent flour on the Buhler mill. Bread was baked by a formula described by Fifield *et al.* (3) using 100 g. of flour, 2.0 g. of compressed yeast, 1.5 g. of salt, 5.0 g. of sugar, 0.25 g. of malted wheat flour, 3.0 g. of shortening, 4.0 g. of nonfat dry milk solids, and varying amounts (0 to 4 mg.) of potassium bromate for

each loaf. The amount of bromate used was adjusted to produce the maximum loaf value. In most instances the loaf having the greatest volume also had the best grain, texture, and crumb color. The protein content of the wheat and the flour was determined by the Kjeldahl method.

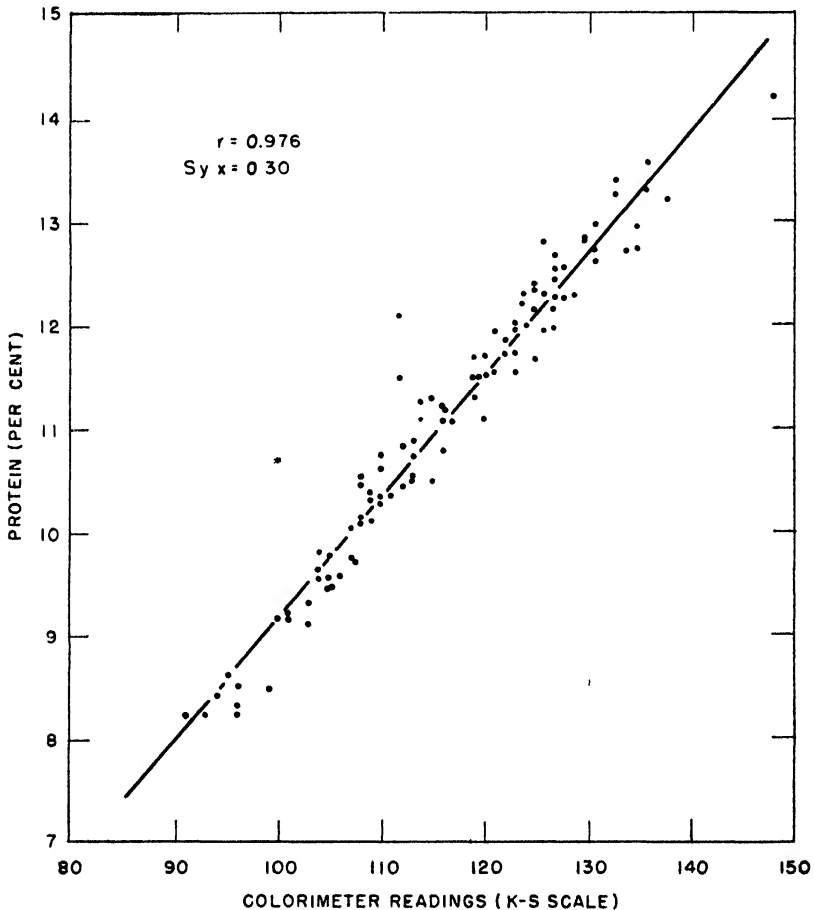


Fig. 3. Correlation of biuret and Kjeldahl protein values of flour milled from hard red winter wheat.

Results

All values presented have been calculated to a 14% moisture basis. Intercorrelations of the different sets of values are shown in scatter diagrams. In Figs. 2, 3, 4, and 5 it may be seen that biuret and Kjeldahl protein values are closely correlated. There is no significant difference, statistically speaking, between the slopes or the positions

of the three lines representing classes of wheat. Obviously the positions of the lines are significantly different from the position of the line representing flour (see Fig. 6).

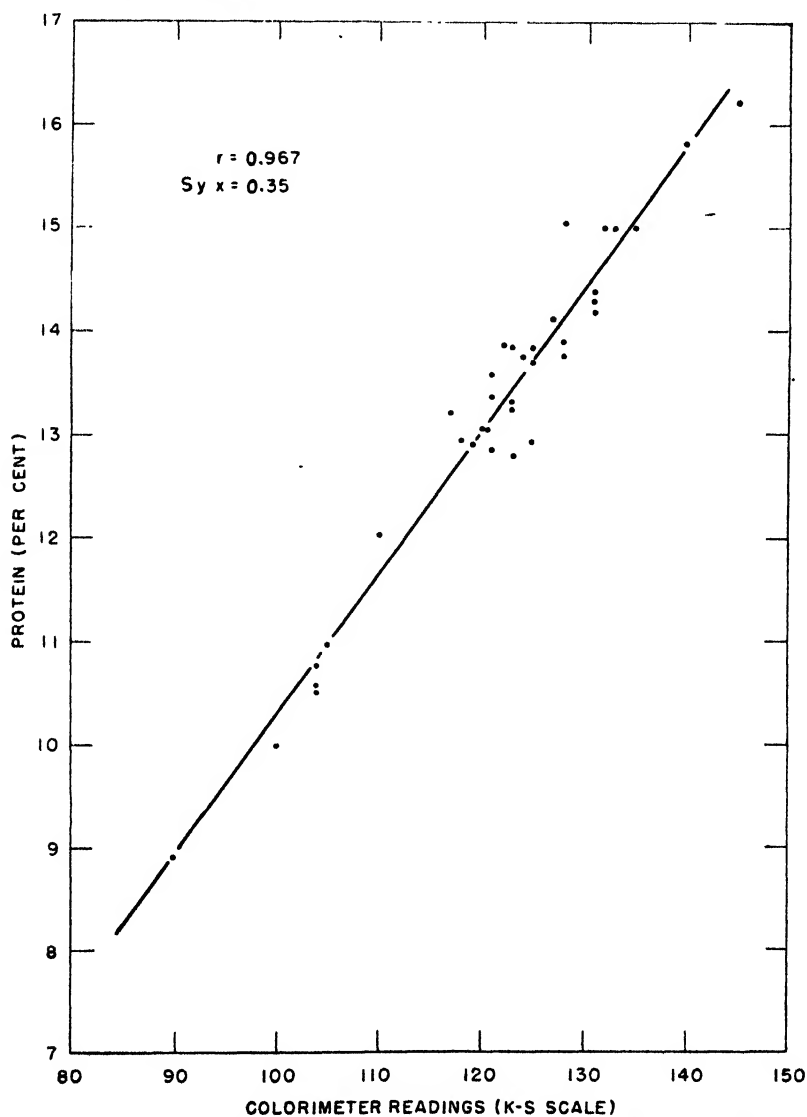


Fig. 4. Correlation of biuret and Kjeldahl protein values of hard red spring wheat.

As previously mentioned, the biuret test as here applied does not directly measure all of the protein contained in wheat or flour. The proportion of protein peptized is greater in flour than in wheat as is

shown by the relatively higher biuret values. In testing 20 of the samples of wheat and corresponding flour, protein was determined in aliquots of the clear centrifugates. Percentages of the total protein represented by the peptized protein were calculated. The averages of these proportions, expressed as percentages, were 85.6 for wheat and 96.8 for flour. Differences in the relative amounts of protein peptized probably account for the differences in slope and position of the regression lines for wheat and for flour, respectively, and may account for the slight differences observed in slopes and positions of regression lines for the three classes of wheat.

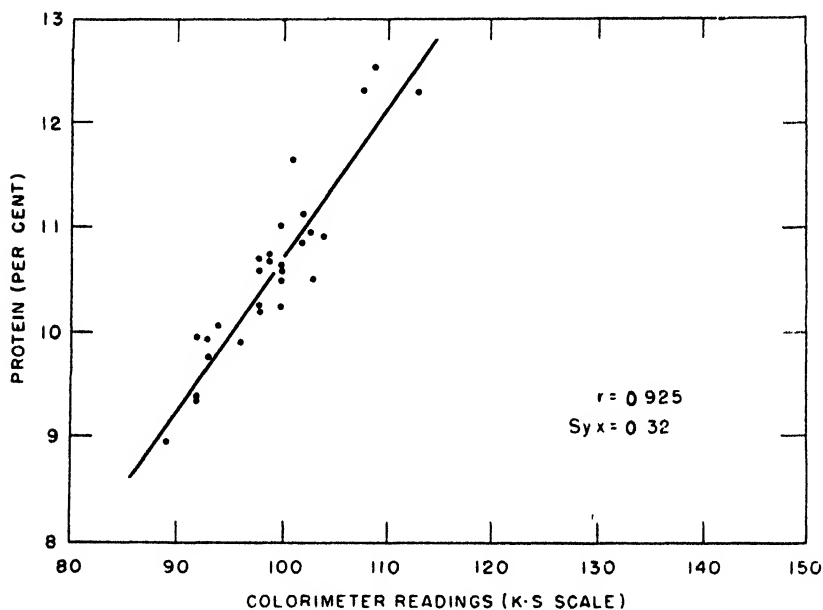


Fig. 5. Correlation of biuret and Kjeldahl protein values of hard white wheat.

Additional evidence that the biuret test compares favorably with the Kjeldahl procedure as a measure of protein content is found in the similarity of correlations with loaf volumes as shown in Figs. 7, 8, 9, and 10.

Reproducibility. Most of the biuret color determinations were made singly. Slightly better correlations would probably have been obtained by the use of the averages of duplicate determinations. In order to demonstrate that good agreement of duplicates can be obtained by this procedure, 22 of the determinations were repeated. As shown in Table II, the average difference between duplicate values obtained is only 0.8 scale unit. Evaluated in terms of protein, this

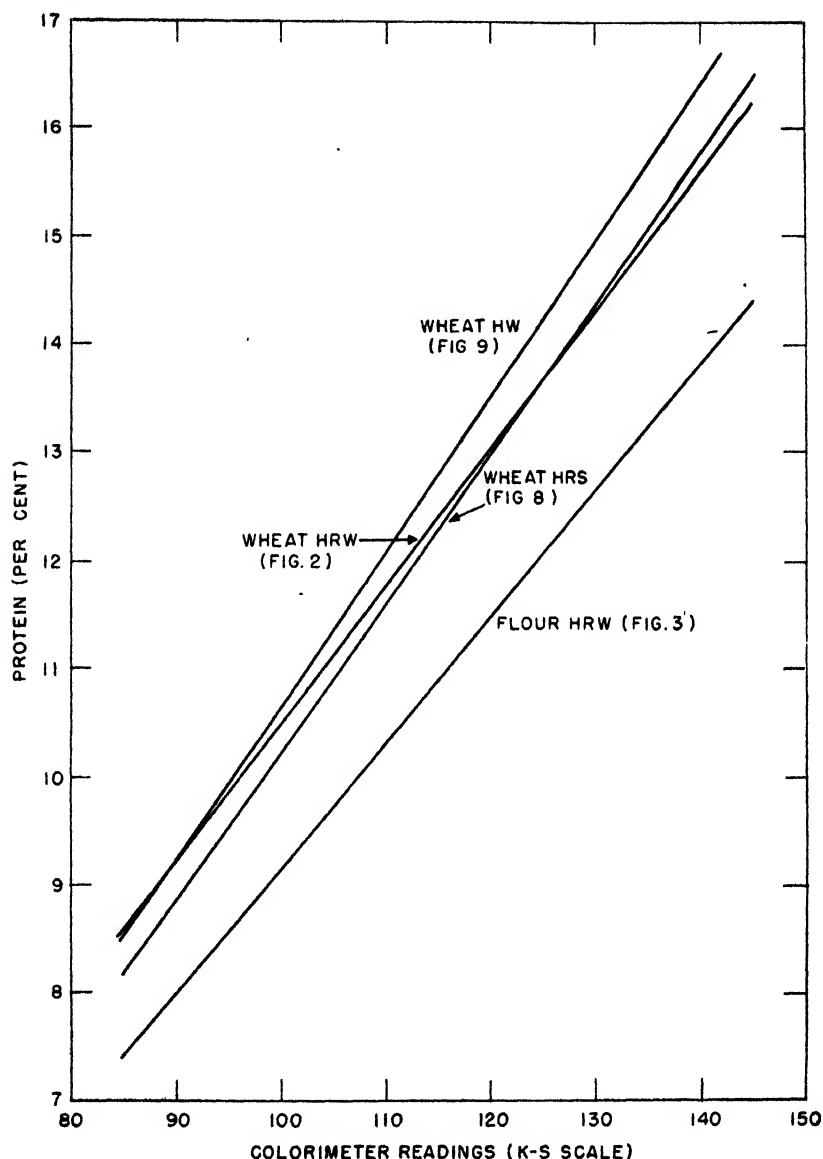


Fig. 6. Regression lines of biuret and Kjeldahl protein values of the three classes of wheat and flour milled from hard red winter wheat.

difference is approximately 0.1%, which compares favorably with the agreement normally expected from the Kjeldahl or other standard procedure.

Application. It is suggested by the data presented in this study

that biuret values may be expressed either (1) as total (Kjeldahl) protein or (2) as protein peptized by dilute alkali. Biuret values expressed in terms already familiar might be more acceptable to cereal workers but could hardly be used interchangeably with values ob-

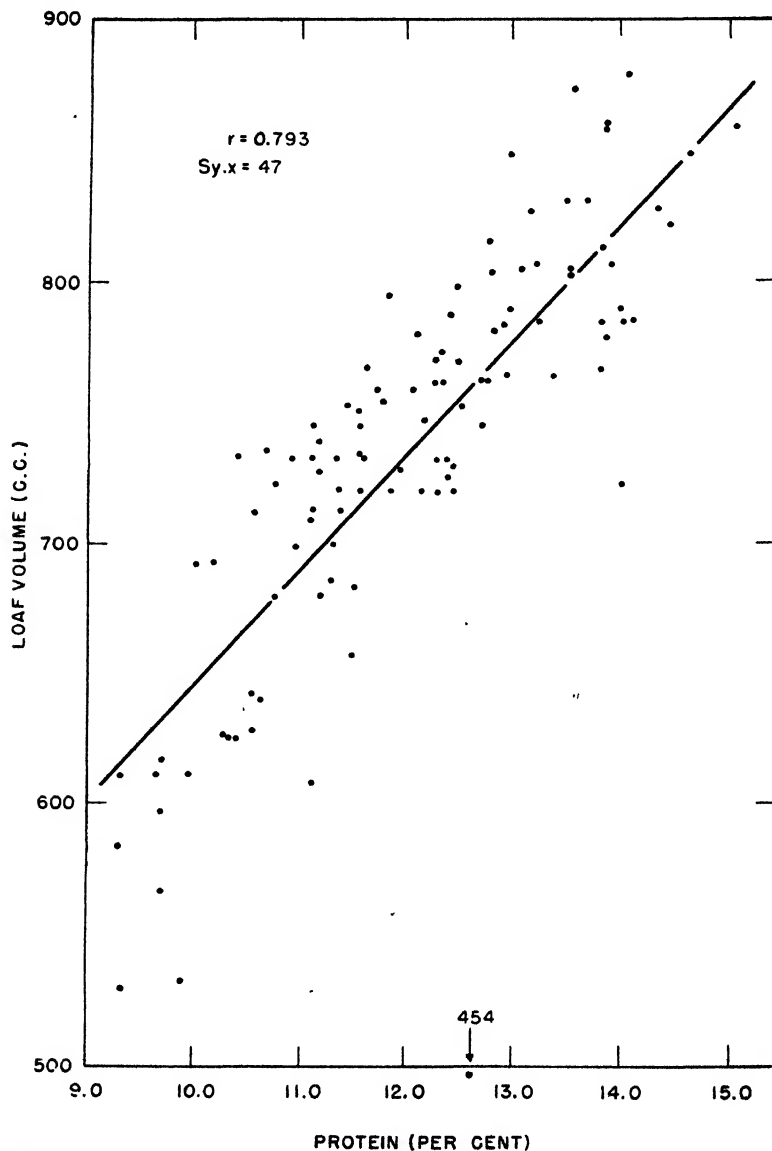


Fig. 7. Correlation of Kjeldahl protein values of hard red winter wheat and loaf volumes.

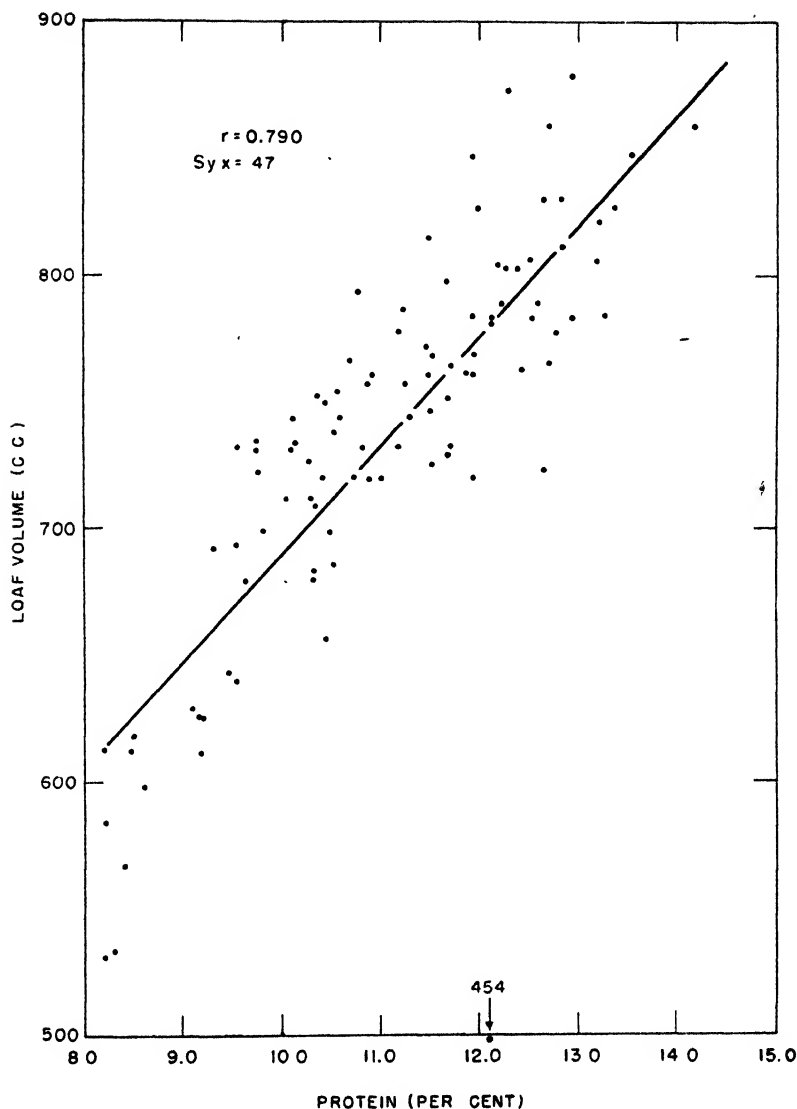


Fig. 8. Correlation of Kjeldahl protein values of flour milled from hard red winter wheat and loaf volumes.

tained by the Kjeldahl method. The values are more accurately expressed as protein peptized by dilute alkali and might be called "Biuret gluten" or some similar name. Conversion of colorimeter scale readings to "Biuret gluten" values would require but one calibration for both wheat and flour.

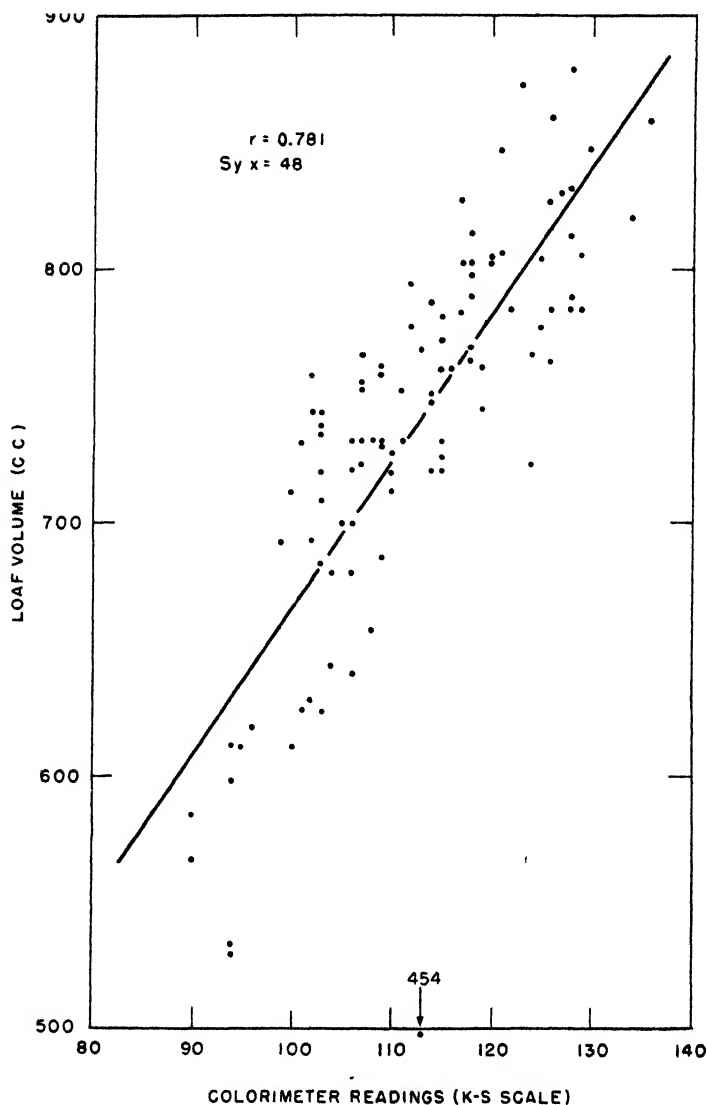


Fig. 9. Correlation of biuret values of hard red winter wheat and loaf volumes.

In general, the scale readings of different colorimeters are not interchangeable, so that each instrument must be separately calibrated. This may be done by determining biuret and protein values of suitable test samples. There may be 30 or more samples of wheat or flour

selected so that the protein values are uniformly distributed over the usual range. Protein values may be determined by the Kjeldahl method, either as total protein or as peptized protein ("Biuret gluten") as desired. From the biuret and protein values a calibration chart

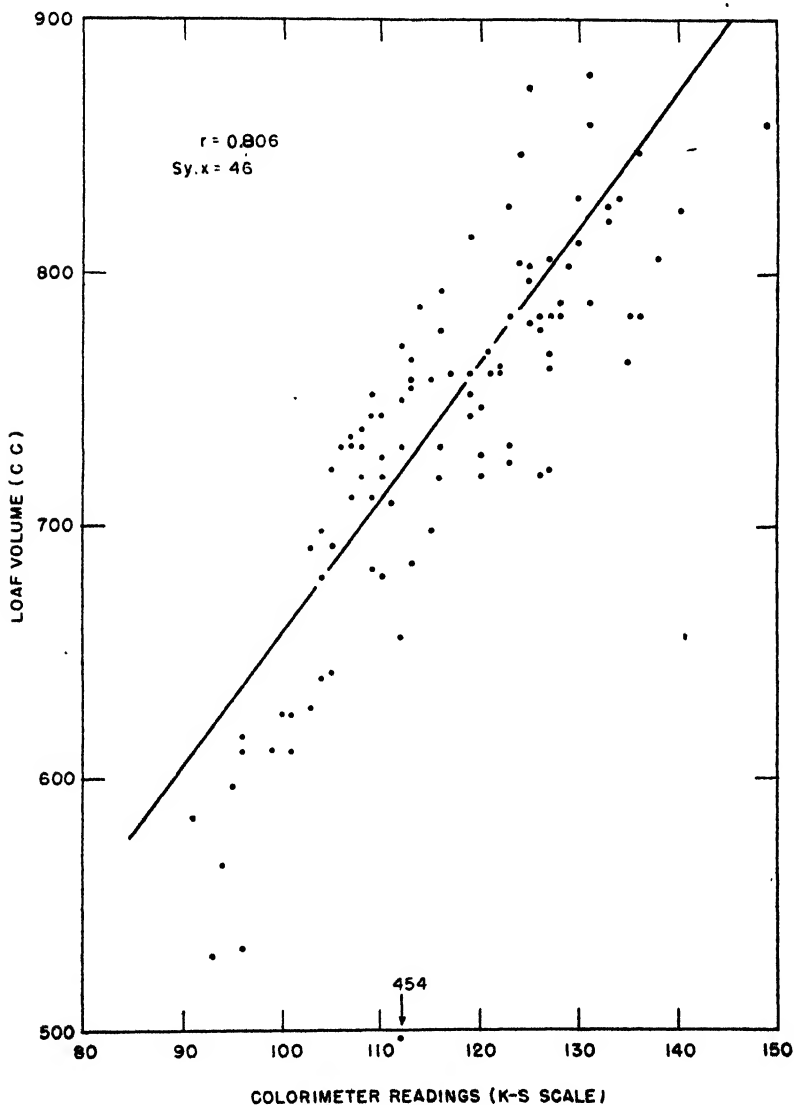


Fig. 10. Correlation of biuret values of flour milled from hard red winter wheat and loaf volumes.

TABLE II
COMPARISON OF DUPLICATE BIURET DETERMINATIONS

KLETT-SUMMERSON SCALE		
"A"	"B"	Difference
128	127	1
126	126	0
148	148	0
119	120	1
129	131	2
120	121	1
105	106	1
105	105	0
125	125	0
100	102	2
112	113	1
131	133	2
106	106	0
107	107	0
136	137	1
126	128	2
135	135	0
134	135	1
128	129	1
97	97	0
105	107	2
102	102	0

Average difference is 0.8 scale unit, equivalent to 0.1% protein.

TABLE III
SUMMARY OF STATISTICS

KJELDAHL PROTEIN CORRELATED WITH BIURET COLOR					
		Flour	Wheat		
		HRW	HRW	HRS	HWb
Corr. coef.	r	0.976	0.962	0.967	0.925
Std. error of estimate	$S_{y \cdot x}$	0.30	0.38	0.35	0.32
	N	100	100	36	28

LOAF VOLUMES CORRELATED WITH:					
		Biuret color		Kjeldahl protein	
		Flour	Wheat	Flour	Wheat
Corr. coef.	N 100				
Std. error of estimate	r	0.806	0.781	0.790	0.793
	$S_{y \cdot x}$	46	48	47	47

(such as Fig. 2), table, or formula may be derived for converting colorimeter values to protein values.

Acknowledgments

The author wishes to acknowledge the assistance of Tyler Hartsing, Technologist, Grain Branch, Production and Marketing Administration, who milled the flours; of C. C. Fifield, Baking Technologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, under whose direction the baking tests were made; of Mary Stutzman, Scientific Aide, Grain Branch, Production and Marketing Administration, who performed the protein tests; of Dr. Lawrence Zeleny, Chief, Standardization Research and Testing Division, Grain Branch, Production and Marketing Administration, who contributed many helpful suggestions during the study and preparation of the manuscript; and of Dr. Thora Hardy, Technologist, Grain Branch, Production and Marketing Administration, who translated the German references cited.

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STUDIES ON BREAD STALING IV. EVALUATION OF METHODS FOR THE MEASUREMENT OF CHANGES WHICH OCCUR DURING BREAD STALING¹

C. W. BICE² and W. F. GEDDES³

ABSTRACT

A modified Farinograph technique and an empirical crumbliness test have been developed for measuring changes that occur in bread as it stales. The Farinograph method comprises measurement of the decrease which occurs in the consistency of crumb "dough" at constant moisture (62.5%); the crumbliness test involves determining the percentage of crumb which passes through a gyrating sieve (0.25 in. mesh) under controlled conditions.

Crumb "softness" (deformation under constant load), swelling power, soluble starch, Farinograph consistency and crumbliness values on a uniform lot of commercial white bread over a 72-hour period, when interpreted in the usual manner, appear to change at different rates as bread stales. The crumbliness values showed the greatest over-all change followed by crumb "softness," the ratios of the highest to the lowest values being 7.1, and 4.2, respectively, as compared with ratios between 1.3 and 1.9 for the other measures. Crumb "softness," Farinograph and swelling power values decreased in a curvilinear manner with time.

Crumb "softness" curves assume the shape of an equilateral hyperbola and cannot readily be interpreted as indices of staling since their slopes are complicated functions of the rate of change with time and of the original softness of the bread. Many conclusions which have been drawn in the literature from crumb "softness" measurements, the most widely used method for following the changes in bread as it stales, may well be erroneous. On the other hand, crumb "firmness" readings (load required to produce a given compression) increase in nearly linear fashion over the customary three- or four-day period of measurement and their slopes are simple direct functions of the relative rates of change in firmness. Crumb "firmness" can be measured with the Baker compressimeter with more precision than crumb "softness."

Fresh, white bread crumb did not obey Hooke's law when compressed and exhibited appreciable plastic flow; as the bread staled the relation between load and deformation became more nearly linear up to higher and higher loads. As a consequence, the choice of the fixed load or fixed compression employed in crumb "softness" or crumb "firmness" measurements will influence the apparent rate of change in these properties.

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The data in this paper are to be included in a thesis to be submitted to the University of Minnesota by C. W. Bice in partial fulfillment of the requirements for the Ph.D. degree.

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Staleness, as it applies to bread, is a generic term covering a number of ill-defined changes that occur in bread as it ages. Consumers judge the staleness of bread by direct perception and their subjective estimates probably represent an unconscious integration of many properties. Any physical or chemical measurement would appear to be, at best, only one factor in the subjective estimate, and the relative merits of various objective measurements can only be properly evaluated by a suitably trained and calibrated test panel.

The extensive laboratory and technological researches which have been carried out on the problem of bread staling have been reviewed recently by Geddes and Bice (14) as an aid to investigations supported by the Quartermaster Food and Container Institute for the Armed Forces. Since the staling of bread represents a complex transformation which is not clearly understood, it has been customary to evaluate the degree of staleness by measuring one or more of the several progressive changes which occur in ordinary bread during aging. The crumb changes which have been measured most frequently are the familiar decreases in softness, swelling power and soluble starch. Since the most obvious change in bread during staling is the development of a relatively rigid crumb structure and as the softness of bread appears to be the criterion used by the consumer as an index of freshness, measurement of the compressibility of the crumb has been the procedure most commonly employed for following the staling process.

While relatively few workers have used more than one or two of the available methods in a particular study, there are indications in the literature that the various methods do not give the same relative results (12, 16, 18).

Preliminary to an investigation of the biochemical changes which occur in bread during aging, a critical study was made of the most promising methods for evaluating gross changes in the physical properties of bread. During the course of these researches, a modified Farinograph method and an empirical crumbliness test were developed and compared with the results of compressibility, swelling power and soluble starch procedures on the same lot of bread. A careful examination was also made of the relative merits of the measurement of crumb "softness" (deformation under constant load) and crumb "firmness" (load required to produce constant deformation). These investigations are reported in the present paper.

Modified Farinograph Technique for Following the Staling of Bread Crumb

Fuller (13) investigated the use of the Farinograph as a convenient measure of the swelling power of the crumb, one of the properties

which has long been used as an index of the changes which occur during staling. In his first experiments, he found that when known weights of bread crumb and water were mixed in the Farinograph, the arbitrary consistency figure was lower for stale than for fresh bread. A subsequent modification consisted of titration of a given quantity of bread crumb with water in the Farinograph to give a standard consistency of 500 Brabender units and recording of the total moisture content. As the bread staled, less water was required to yield a "dough" of this standard consistency, and the decrease in absorption was taken as a measure of the staling rate.

Studies with this method made here showed that the results were markedly influenced by the rate at which the water was added, and the replicability of the test was poor. It was noted that very fresh bread crumbs, even without the addition of water, would mix to form

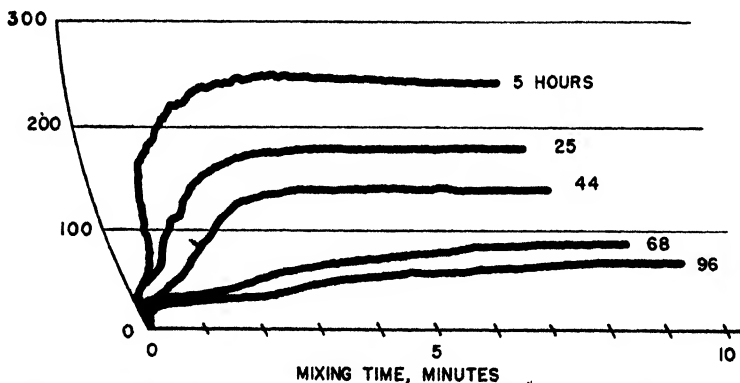


Fig. 1. Curves showing the progressive decrease in Farinograph maximum consistency values of ordinary bread crumb during storage.

a dough in the Farinograph. The recorded curve was smooth and gave a definite, reproducible maximum. Upon addition of water and mixing, stale crumb also formed a dough and gave curves showing a lower maximum consistency than the crumb doughs made from fresh bread.

The minimum amount of water necessary to form a dough with very stale crumb was determined and used as a standard total moisture content for both fresh and stale bread crumb. The modified Farinograph method finally developed was as follows:

A 30 g. sample of crumb (dry weight basis) is made up to 62.5% moisture content with distilled water in the 80 gram Farinograph bowl, and mixing at 30°C. begun immediately. Within 5 to 10 minutes a maximum consistency is reached and is recorded in Brabender units.

Figure 1 illustrates the typical progressive decrease of maximum Farinograph consistency values of bread crumb during staling at room temperature.

Table I illustrates the change in maximum Farinograph readings when the above method was used to follow changes during storage in commercial white bread, laboratory-prepared white bread and canned white bread. The data for all samples indicate a progressive decrease in crumb-dough consistency over the period studied.

TABLE I
CHANGES IN FARINOGRAPH CRUMB-DOUGH CONSISTENCY OCCURRING IN
SEVERAL WHITE BREADS DURING STORAGE IN AIR-TIGHT
CONTAINERS AT LABORATORY TEMPERATURE

Commercial		Laboratory-prepared		Canned	
Age of bread	Farinograph reading	Age of bread	Farinograph reading	Age of bread	Farinograph reading
<i>hrs.</i>	<i>B.U.</i>	<i>hrs.</i>	<i>B.U.</i>	<i>hrs.</i>	<i>B.U.</i>
3.3	340	5.0	240	3.0	260
7.0	350	5.2	240	26.0	160
8.0	330	25.0	180	49.0	180
11.0	300	44.0	140	49.5	130
18.2	270	44.2	140	97.5	100
19.3	270	68.0	90	98.0	100
24.0	250	96.0	80	147.0	60
48.0	210			267.0	60
72.0	180			361.0	50
96.2	150				

The possible effect of pH variations on the maximum Farinograph consistency of crumb doughs at constant moisture was investigated by adjusting the pH of the doughs to various levels with solutions of hydrochloric acid and sodium hydroxide. The maximum consistency readings were virtually uninfluenced over a pH range of 1.6 to 7.4. Since the pH of bread normally lies between 5.0 and 5.6, these limited experiments indicated that variation in pH would not be an interfering factor in the application of this technique to measurements made on ordinary bread.

Development of a Method for Measuring Crumbliness

It has long been known that the tendency of bread to crumble when sliced increases upon staling and Selman (24) has recently developed an arbitrary method of measuring the crumbliness of cakes. The method involves the vertical oscillation of cake crumb in a cylindrical device fitted with a sieve, and determination of the quantity of crumb which falls through the sieve. Preliminary studies in this laboratory indicated the possibility of utilizing the gyrator sifter of an

Allis-Chalmers experimental mill in developing an empirical crumbliness test. The effect of crumb surface, sieve mesh opening and of the speed and time of sifting were investigated and the empirical crumbliness test described below was evolved.

A square box with tight fitting slide-on cover and removable sieve supported within, was designed to fit into the gyratory bolter of an Allis-Chalmers experimental mill. The inside dimensions of the box

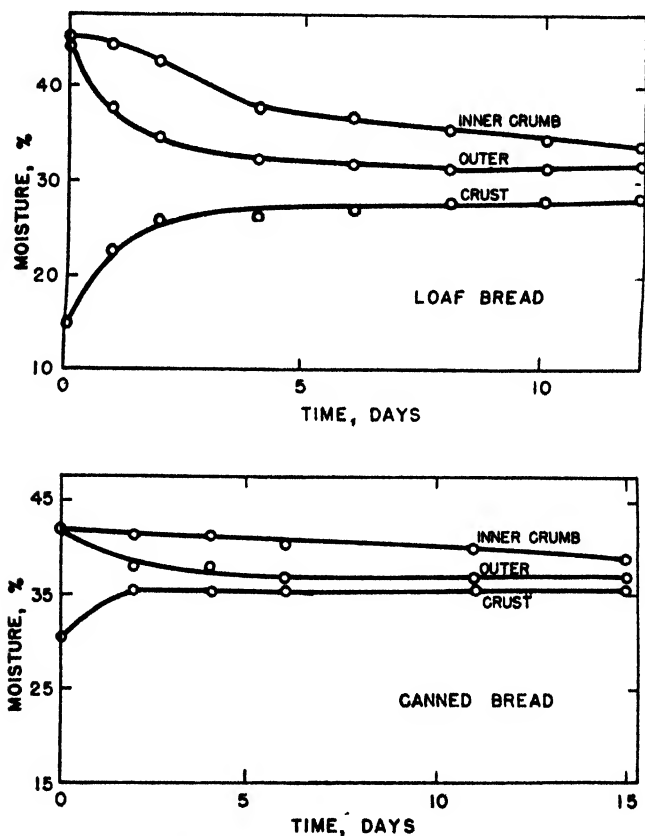


Fig. 2. Curves showing the change in moisture content of crust, inner crumb and outer 0.5 inch of crumb of ordinary white loaf bread and canned white bread during storage at room temperature

were $3 \times 13 \times 13$ in. The lid was held in place by metal flanges along two sides of the box. The effective sieve area was one square foot, and the mesh, $\frac{1}{4}$ by $\frac{1}{4}$ in. Bread crumb slices $1\frac{1}{4}$ in. square and 0.5 in. thick were used for the determination of crumbliness. A 50 g. sample ("as is" basis) of the crumb slices was placed in the covered sifter box and shaken at 270 r.p.m. for 10 minutes in a room maintained at approximately 25°C. and 75% relative humidity. The

crumb passing the sieve was collected, dried in a forced draft oven at 130°C. for one hour and weighed. Data are reported as per cent of the original sample (dry basis).

Distribution of Moisture in Bread Crumb

When bread is stored, it seems quite likely that the crumb loses moisture to the relatively dry outer crust; such a decrease in crumb moisture would set up gradients which might be of sufficient magnitude to influence the compressibility and crumbliness. That such a moisture gradient does exist was shown by determinations of the moisture content of the crust, outer 0.5 in. of crumb, and inner crumb of ordinary white bread (experimental loaves baked from 150 gram doughs) and of canned bread (No. 2½ tins) made by the regular army formula. Measurements were made after allowing the loaves to stand for 12 to 15 days in air-tight containers at room temperature. The results presented graphically in Fig. 2 show that immediately after baking, the inner and outer crumb have the same moisture content whereas the crust is considerably drier; upon storage, the outer crumb loses moisture to the crust thereby setting up a moisture gradient in the crumb which still existed after a period of 12 to 15 days.

Comparative Rates of Changes in Various Properties of Bread

After experience had been gained with various procedures, a comparative study was made of the compressibility, swelling power, "soluble starch" (water-extractable polysaccharides), Farinograph and crumbliness methods for measuring the gross changes which occur in bread as it ages.

For this study, 60 1.5 lb. loaves of white bread were obtained direct from a commercial bakery and allowed to cool to room temperature. To lessen the possibility of the establishment of a moisture gradient, during storage, the crust and outer 0.5 inch of crumb were removed from half of each loaf and the remaining crumb was then shredded by a 10-second treatment in the Waring Blendor, combined and sealed in cans to provide a representative uniform sample for Farinograph, swelling power and soluble starch determinations. The remaining halves of each loaf were sliced and the crust and outer 0.5 in. of crumb removed as before. Alternate slices were then separated and stored, at room temperature in a definite order for compressibility and crumbliness determinations, respectively. The slices for the crumbliness study were further cut into 1¼ inch squares before being sealed in airtight containers. Measurements by the various methods were made 3, 7, 11, 18, 24, 48, and 72 hours after baking.

Crumb compressibility values were obtained by means of a Baker compressimeter using a pressure plate 1.0 in. in diameter and crumb slices 0.5 in. thick and three in. square. The compression in mm. under a 20 g. stress was recorded.

Swelling power was determined by the modified method of Schoch and French (1, 22).

"Soluble starch" was precipitated by means of absolute ethanol from the supernatant crumb extract obtained during the swelling power determination. The procedure was similar to that applied by Schoch and French (22). Data were recorded as per cent dry matter obtained from the original crumb (dry basis).

Maximum Farinograph consistencies and crumbliness were determined according to the modified techniques already described in this paper.

TABLE II
STALENESS CHANGES IN COMMERCIAL WHITE BREAD
AS MEASURED BY SEVERAL METHODS

Age of bread	Crumbliness	Compressibility	Farinograph consistency	Swelling power	Soluble starch
<i>hrs.</i>	<i>%</i>	<i>mm.</i>	<i>B.U.</i>	<i>grams</i>	<i>%</i>
3	7.6	2.1	340	4.25	4.5
7	10.5	2.1	340	4.14	—
11	13.1	2.0	300	3.93	4.0
18	20.2	1.1	275	3.72	3.8
24	23.4	0.8	250	3.53	4.0
48	39.4	0.6	205	3.24	3.6
72	54.1	0.5	175	3.02	3.4
Ratio of highest to lowest value	7.1	4.2	1.9	1.4	1.3

The results summarized in Table II represent the means of duplicate determinations, with the exception of the compressibility figures which were the means of readings on 15 slices of bread.

This experiment was conducted several years ago and the data are presented to demonstrate not only the comparative results but also some of the apparent difficulties and disadvantages underlying their use and interpretation.

Interpreting these data in the usual manner would lead to the conclusion that the crumbliness values showed the greatest over-all change and that crumb "softness," Farinograph, swelling power and soluble starch values showed progressively less over-all changes. This would suggest that the different methods do not indicate the same extent of staleness over the 72-hour period. Further, when the changes for each time interval expressed as a percentage of the total change for each respective method were plotted against time (Fig. 3)

the various methods also appeared to show different rates of staling. Thus, 80% of the total change in compressibility value over the 72-hour period occurred within the first 24 hours and thereafter the change was very gradual. Crumbliness on the other hand, showed very nearly a linear change with time.

The Farinograph consistency and swelling power methods gave results which changed in a curvilinear manner with time. The curve for the soluble starch data is not shown on the graph, but it was found to lie slightly above the swelling power curve and to be curvilinear.

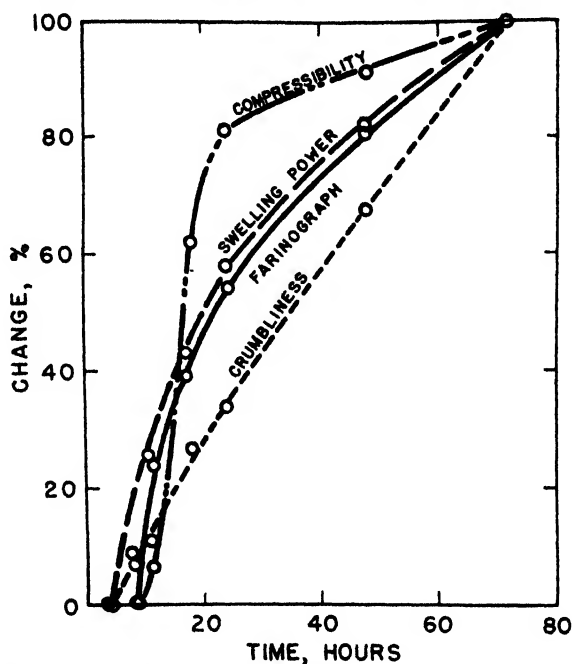


Fig. 3. Curves showing the per cent change in several properties of bread crumb during staling.

As will be shown later, the comparison of staling data is not as simple as would be indicated by the usual treatment above.

Measurement of Crumb Compressibility

The development of a relatively rigid crumb structure as bread ages provides a mechanical basis for following and recording the staling process in numerical terms. Several devices for measuring the compressibility of bread crumb have been employed by different workers (2, 3, 4-8, 9, 16, 18-21, 23, 25). Of these, the Baker compressimeter (1, 21) was especially designed for measuring the compression which occurs when a flat disc is pressed against the surface

of the crumb under a given load; since the force is progressively applied by means of a lever and windlass operated by a synchronous motor, a series of force and deformation values can be read from the scales which are provided.

Crumb hardness changes in bread crumb have been measured in two ways—either by noting the force required to give a standard compression value; or by observing the compression obtained upon application of a standard force. In the first instance, greater and greater loads are required with time to produce a standard deformation, and it is logical and convenient to call the values "firmness" readings. However, in the second method, smaller and smaller deformation readings are obtained with time for a given standard force and they may be termed "softness" data. The vast majority of workers have measured the compression produced by a constant load but recently Favor and Johnston (11) and Thomas (26) have measured the force required to produce a constant deformation in studies of the effects of certain ingredients and other variables on the rate of staling of bread. They found that certain ingredients delayed the rate at which the bread became firm, a conclusion which is at variance with that of Sumner and Thompson (25) who determined the compression produced under a constant load. The relative merits of crumb "firmness" and "softness" measurements thus appear to require careful examination.

Crumb compressibility values are naturally influenced by such factors as the moisture content of the crumb and its distribution and by the size and thickness of the cell walls. The crumb grain, and also the moisture content, may vary in different sections of the loaf and it is generally realized that the replicability of the test is rather poor although quantitative data are limited to the studies of Freilich (12) with bread and of Noznick and Geddes (17) with cake.

Platt (19) subjected bread crumb, three hours out of the oven, to several different loads and found that the deformation varied directly with the magnitude of the load; that is, it followed Hooke's law and behaved as a perfectly elastic body. The question whether bread crumb follows Hooke's law is of such theoretical and practical interest that further investigations of the relation between stress and strain should be made. If this law holds for bread, the crumb "firmness" can readily be computed from crumb "softness" data and vice versa; moreover, these results could be expressed in terms of a modulus of elasticity, a constant which is characteristic of the material and entirely independent of the stresses (or strains) employed in carrying out the measurements.

It must be emphasized that cereal chemists and physicists use the term "compressibility" in an entirely different sense. In physics, compressibility is the reciprocal of the bulk modulus, the modulus of elasticity which applies only in cases where the force or pressure is brought to bear equally on all sides, or over the whole surface of the test object. The bulk modulus is not applicable to bread compressimeter measurements where the pressure or force is applied only to two faces of the bread section. The most appropriate modulus of elasticity in such cases, provided Hooke's law is followed and shearing does not occur, is Young's modulus (the modulus of stretch) in which by definition, equal force is exerted at opposite ends of the test object. Young's modulus = force per unit area/change in length per unit length; that is,

$$Y = \frac{F/A}{e/L} = \frac{FL}{Ae},$$

where, in the case of bread, F is the force applied; A , the effective area of the pressure plate; e , the decrease in slice thickness; and L the initial slice thickness. Young's modulus, stress per unit strain, has been used in the cereal chemical literature (17, 19) as a modulus of compressibility. Since crumb "firmness" readings are force values they are directly proportional to Young's modulus or to the modulus of compressibility as defined in the cereal chemical literature.

Theoretical and experimental observations concerning the various aspects of compressibility methods for following bread staling are presented in the sections which follow.

Crumb "Firmness" vs. Crumb "Softness" as Measures of Staling Rate

An indication of the most appropriate method of expressing and interpreting crumb compressibility data may be obtained from a theoretical consideration of the behavior of bread under compression.⁴ For the sake of simplicity, it will be assumed that bread satisfies Hooke's law when compressed.

Let F = the actual force applied to the pressure plate of the compressimeter, and let σ = surface area of pressure plate, d_0 = original thickness of crumb, d = actual thickness of crumb under load. Then stress, $Z = F/\sigma$; and strain, $\epsilon = (d_0 - d)/d_0 = \delta/d_0$.

If Hooke's law is satisfied

$$Z = E(t) \cdot \epsilon. \quad (1)$$

The modulus, $E(t)$, is similar to Young's modulus and in order to take care of changes due to staling it is assumed to be a function of

⁴ The authors are indebted to Professor Andrew Hustrulid, Physicist, Division of Agricultural Engineering, University Farm, for the theoretical treatment presented here.

time. If the isometric ⁵ ($\epsilon = \text{constant}$) is a straight line, then $E(t)$ is a linear function of t and may be written

$$E = E_1 t + E_2. \quad (2)$$

E_2 is a measure of the initial condition of the crumb. If E_1 is defined as a measure of the rate of staling, it can be found easily by experimentation. It is simply $1/\epsilon$ times the slope of the isometric.

$$\frac{1}{\epsilon} \left(\frac{\partial Z}{\partial t} \right)_{\epsilon} = \frac{1}{\epsilon} \frac{\partial}{\partial t} [(E_1 t + E_2)\epsilon] = E_1. \quad (3)$$

In practice, crumb "firmness" curves represent a plot of the force applied to the compression plate to produce a constant compression (δ) as a function of time. Equation (1) may be written:

$$F = \frac{\sigma}{d_0} E(t) \delta, \text{ when } F = \text{"firmness" or load.} \quad (4)$$

The slope of the "firmness" curve is therefore proportional to the rate of staling as defined above, since

$$\left(\frac{\partial F}{\partial t} \right)_{\delta} = \frac{\sigma \delta}{d_0} E_1 = K E_1.$$

The rates of staling of different breads can be evaluated easily by comparing the slopes of the "firmness" curves which are obtained for constant values of σ , d_0 and δ .

When δ is measured as a function of t for a constant load, F , it is a measure of crumb "softness" and the question arises whether the rates of staling of different breads can be obtained by comparing the slopes of the "softness" curves.

From equation (4)

$$\delta = \frac{d_0 F}{\sigma E(t)} = \frac{d_0 F}{\sigma (E_1 t + E_2)} \quad (5)$$

and

$$\left(\frac{\partial \delta}{\partial t} \right)_F = \frac{-d_0 F}{\sigma} \frac{E_1}{(E_1 t + E_2)^2}. \quad (6)$$

It is obvious from equation (6) that a measure of E_1 for different breads cannot be obtained by comparing the slopes unless they had the same modulus initially; that is, unless E_2 is the same for all breads being compared. Even then the task would be difficult and the results uncertain because the slopes are constantly changing with time. Within the limits of the assumptions that the force, F , is proportional

⁵ The isometric is the "curve" representing the value of Z as a function of time for conditions of constant strain.

to the compression, δ , and that the rate of staling, E_1 , is constant, it must be concluded that the relative slopes of "firmness" curves provide the only satisfactory measure of comparing staling rates from compressibility data.

The question whether one should use "firmness" or "softness" units is therefore not purely an academic one, especially when attempts are made to reach conclusions regarding relative staling rates of two or more breads by a mere visual examination of the curves, the practice which has been followed by most workers in this field. "Softness" curves drawn through a sufficient number of points ordinarily tend to approximate an equilateral hyperbola, whereas, as will be seen later

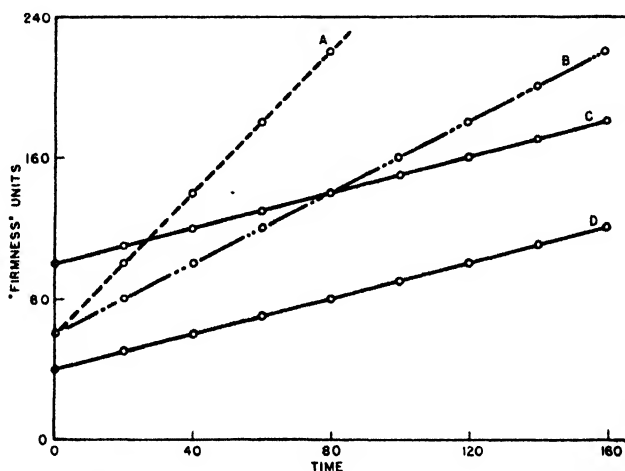


Fig. 4. Ideal "firmness" data plotted against time. General equation for the curves is: $y = 2mt + b$, where y = firmness units, t = time, b = y intercept or initial firmness reading, 2 = y scale adjustment since there are twice as many firmness units per linear dimension of ordinate as there are time units per abscissa, and m = the slope, or rate of firmness change. Slopes are as follows: $m_A = 1$, $m_B = 0.5$, $m_C = 0.25$, and $m_D = 0.25$.

in this paper, "firmness" curves are more nearly linear over the three- or four-day period during which staling is normally followed; that is, "softness" is approximately an inverse function of time whereas "firmness" is approximately a direct function of time. "Softness" is approximately the reciprocal of "firmness," the relationship between them being constant only if the original stress-strain data conform to Hooke's law.

The implications of the preceding theoretical treatment of the relative utility of the two methods of expressing the results of compressibility measurements may be demonstrated graphically by plotting hypothetical staling data in which it is assumed that crumb "firmness" increases in a perfectly linear fashion with time. Four

"firmness" curves, the equations for which are known and so chosen as to demonstrate the various characteristics which one hopes to compare by inspection in practical studies, are plotted in Fig. 4. Curves C and D differ in height but have the same slope; thus, "bread" D is "softer" than "bread" C but the rates of change are the same. Curves A and B, on the other hand, have the same original "softness" but A changes more rapidly than B. The reciprocal values of these "firmness" data are plotted in Fig. 5; the four "softness" curves (A', B', C', D'), correspond to the "firmness" curves (A, B, C, D, Fig. 4). These reciprocal values give curves which are displaced equilateral hyperbolae, the shapes of which are characteristic of "softness" curves, appearing in the literature. It is readily seen that the rate of staling

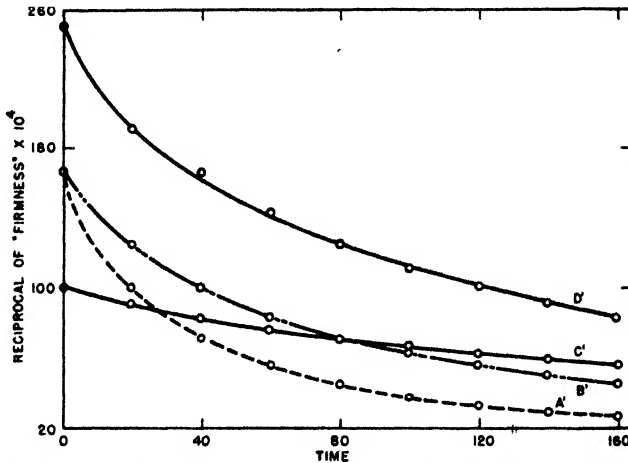


Fig. 5. Ideal "softness" data or the reciprocal of "firmness" of Fig. 4. General equation for the curves is: $S = 10^4/y = 10^4/(2mt + b)$, where S = reciprocal of firmness, t = time, $m_{A'} = 1$, $m_{B'} = 0.5$, $m_{C'} = 0.25$, $m_{D'} = 0.25$, and $b_{A'} = 60$, $b_{B'} = 60$, $b_{C'} = 100$, and $b_{D'} = 40$.

of breads C and D might be evaluated quite differently when "softness" is plotted rather than "firmness." The differences in the original "firmness" of the bread results in a variation in the rate of change in the slope of the "softness" curves which many would erroneously interpret as a reflection of differences in the rate of staling of the two breads. In contrast, the widely divergent "firmness" curves A and B indicate a wide difference in the staling rate of these two breads; whereas the "softness" curves A' and B' (Fig. 5) might lead one by superficial inspection and erroneous reasoning, to conclude that A' and B' are staling at essentially the same rate and that bread B' is merely softer than A'. Closer inspection and calculation of the slopes at successive times, reveals that bread A' apparently stales at

a greater rate than bread B' between 0 and 40 time units; thereafter, the reverse is the case.

The apparent parallel nature of the "softness" curves A' and B' has been the chief misleading factor in interpreting these data and has led to the general but fallacious conclusion that few, if any, adjuncts have any appreciable effect on the rate of staling. Actually all "softness" curves must tend to approach one another gradually as they become asymptotic to a limiting horizontal axis. This asymptotic region of "softness" curves does not represent a true staling limit as has been inferred in the past but rather it is an artificial limit which depends on several factors in addition to the staling rate. This fact appears to explain why Cathcart (6) and others, have objected to crumb "softness" (as well as swelling power and soluble starch values which also decrease with time) as measures of staling, since the greatest changes occur within the first several hours after baking or at a time when bread is still considered fresh by human judges. As indicated by a comparison of curves C and D with C' and D' the initial "softness" reading greatly effects the shape of "softness" curves and such curves may lead one erroneously to think that maximum staleness has been approached merely because the curves level off rather sharply as they tend to become asymptotic. The slopes of "firmness" curves, on the other hand, are not affected by such artificial limits, but depend entirely on the rate of change of "firmness" readings.

The relative complexity of "softness" data, as compared with "firmness" values may be illustrated more precisely by comparing the first derivatives, (or slopes) of the curves shown in Figs. 4 and 5. In the case of the "firmness" data for which the general equation is $y = 2mt + b$, the slope is $dy/dt = 2m$, where b is the initial "firmness" reading, m the slope, t the time and 2 = "y" scale adjustment. In sharp contrast, the slope of the "softness" curves (Fig. 5) having the general equation $S = 10^4/y = 10^4/(2mt + b)$, is constantly changing and is represented by the relatively complex first derivative:

$$\frac{dS}{dt} = - \frac{10^4 \times 2m}{(2mt + b)^2}$$

It is readily seen that the slope of the "softness" curve at any given time is dependent on the initial "softness" reading $10^4/b$, as well as on t and m . The slopes of "softness" curves are so complicated that one cannot be compared directly with another even with appropriate mathematical treatment. In accordance with common scientific practice, it is desirable, whenever possible, to express analytical data in a manner which leads to the most nearly linear relationship.

The preceding discussion is based on hypothetical ideal "firmness" curves, the equations for which were known; but the advantage of utilizing crumb "firmness" rather than crumb "softness" can be shown readily with data from the literature. As an example the crumb "softness" values obtained by Platt (19) are shown in Fig. 6, and the reciprocals of these values are plotted in Fig. 7. The "softness" data yield the well-known crumb compressibility curve, approximating that of an equilateral hyperbola, while the reciprocal values yield a much more nearly linear relationship; indeed considering the errors

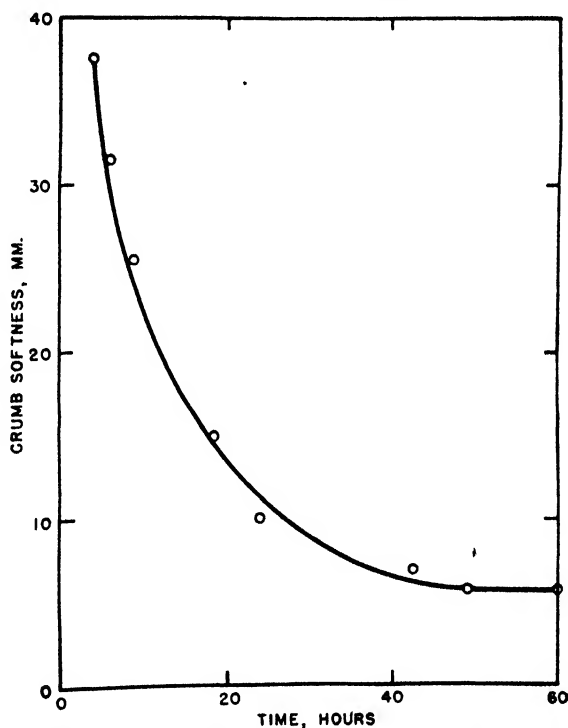


Fig. 6. Change in crumb softness of bread crumb with time. Data of Platt (19).

involved in this technique, the actual relation may be perfectly linear. Similar results were obtained upon the corresponding treatment of the compressibility data of Noznick, Merritt, and Geddes (18).

Also, direct determinations of crumb "firmness" by Crossland and Favor (10) using the Baker Compressimeter have given approximately linear curves when the force readings were plotted against a series of staling times up to 90 hours. Similar results have been consistently obtained by the present authors. Such data were the basis for the assumption in the foregoing theoretical treatment that "firmness"

data showed a linear change with time. It may be concluded that the forces required to yield a standard compression may be utilized directly to indicate differences in the crumb "firmness" of various breads at any time and also as a time index of the rate at which the bread becomes hard over the testing period normally employed. It is not, of course, implied that the crumb "firmness" change would continue to be linear over extended storage periods. In practice, interest in the rate of change is normally confined to the first few days.

It is clear that the conclusions which have been drawn in the literature from "softness" curves which represent (approximately)

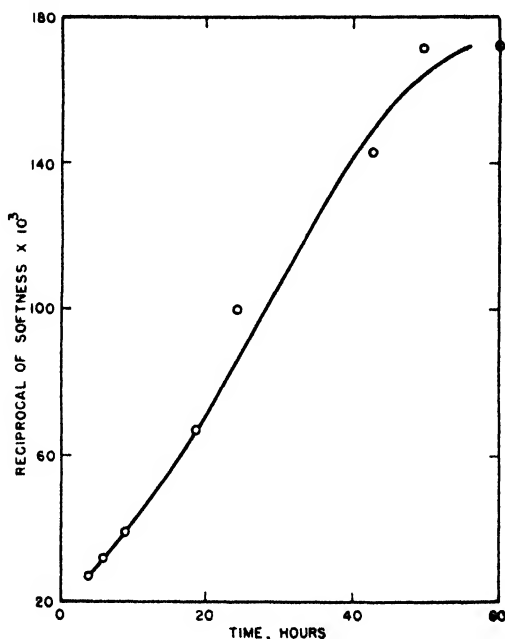


Fig. 7. Change in the reciprocal of crumb softness values (computed from data of Platt shown in Fig. 6) with time.

equilateral hyperbolae may well be erroneous. Similarly, other methods of measuring changes occurring during staling, the results of which markedly decrease with time and which yield similar curves, may also have been subject to erroneous interpretation. The Farinograph consistency data of Freilich (12) for example yield such curves, and the reciprocals show an approximately linear relation with time.

The authors regret that they were unaware of this anomaly when they reviewed the bread staling literature (14), as some of the conclusions may well require revision including those in papers with which one of us (W. F. Geddes) has been associated. A completely contrary

view of the relative merits of the two methods of expressing compressibility data has been recently expressed by Bradley (5).

Stress-Strain Relationships

Many series of force—deformation curves have been obtained in this laboratory for commercial white bread, army canned bread and laboratory-baked bread for various treatments and staling times but a few typical examples will serve to illustrate the general nature of the results. The maximum deformation which can be read with the Baker compressimeter, employed in this laboratory, is 4.0 mm. and it is possible to obtain consecutive deformation readings over a much wider range of forces in the case of canned bread than of ordinary

TABLE III
STATISTICAL CONSTANTS FOR DEFORMATION VALUES OBTAINED BY APPLYING
VARIOUS LOADS TO CANNED BREAD DURING STORAGE
AT ROOM TEMPERATURE¹

Load	Mean ² deformation	Variance		F for between times ²	Standard error (single determination)	
		Between times ²	Within times		Absolute	Per cent of mean
<i>g.</i>	<i>mm.</i>				<i>mm.</i>	
40	0.218	0.029	0.006	4.6	0.078	36.0
80	0.379	0.124	0.016	7.5	0.128	33.9
120	0.527	0.370	0.032	11.5	0.180	34.1
160	0.708	0.820	0.078	10.5	0.280	39.5
200	0.882	1.918	0.141	13.6	0.376	42.6
240	1.116	3.706	0.221	16.8	0.470	42.1
280	1.415	7.825	0.342	22.7	0.587	41.5
320	1.662	13.275	0.516	25.7	0.718	43.2

¹ Fourteen slices were read at ages of 0.92, 1.9, 4.6, and 9.75 days respectively.

² For staling times of 0.92 to 9.75 days inclusive

³ All values are highly significant.

white bread; the canned bread is more dense and contains less moisture so that it is firmer than ordinary loaf bread. The bread made according to the current army formula was sliced 0.5 inch thick, from which one-inch squares were cut and stored in air-tight containers. Deformation values were read on 14 replicate slices at loads from 40 to 320 grams in 40 gram increments for staling times of approximately 1.5 hours, 1, 2, 5, and 10 days. With 1.5-hour-old bread, it was not possible to obtain deformation readings above a 160-gram load. The mean deformation data for each load over all staling times are recorded in Table III together with a summary of a variance analysis of the individual data and the standard errors. The mean deformation values for each load and staling period are plotted in Fig. 8 with the exception of the data for 9.8 days, the curve for which practically

coincided with that for 4.6 days. The differentiation between the deformation values for the various staling times is increased by increasing the load; the F values for the differences in deformation due to staling time increase from 4.6 to 25.7 as the loads are increased from 40 g. to 320 g.

The experimental errors involved in reading deformation values are extremely high in comparison with other analytical determinations employed by the cereal chemist, which emphasizes the importance of employing a large number of replicates and carrying out statistical analyses before drawing conclusions concerning the influence of the variables under study. Part of the experimental error is due to variations in the density of the crumb from the top to the bottom of

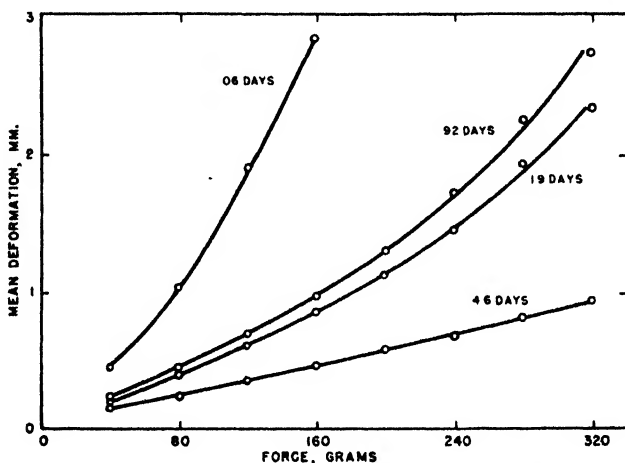


Fig. 8. Mean deformation values for army type canned white bread plotted against forces for various staling times. (Each point is a mean of 14 individual determinations, that is, 14 test slices.)

the can; in the majority of instances the crumb was softest at the center and firmest at the top.

The curves in Fig. 8 indicate that the relation between deformation and load varies for crumb of different ages. A statistical analysis revealed that for the 1.5-hour-old bread, the relation was curvilinear for even small loads. As the crumb aged and became less compressible the relation became more linear up to higher and higher loads. For the bread 0.92 days old the curve was found to be linear between loads of 40 and 160 g., for bread 1.9 days old it was linear between loads of 40 and 200 g., whereas for the bread 4.6 and 9.75 days old, the curves were linear between all loads employed. However, the maximum deformations to which the oldest breads were subjected was less than one mm. and did not approach those for the fresher samples, due to the fact that a load of 320 g. was the maximum for the instrument.

The choice of the force, or deformation, value for following bread staling will obviously influence the apparent staling rate since they will affect the extent to which the results will deviate from Hooke's law; the comparisons will include some values which lie on the approximately linear portion of the stress-strain curve for bread of a given age; at other staling periods the recorded values will represent data from the curvilinear portion.

A similar study was carried out with commercial white bread made without the addition of commercial emulsifiers or softening agents, employing a larger pressure plate and different slice thicknesses in an effort to improve the replicability of the test. Fifty commercial 1.5 lb. loaves were obtained from the same dough baked in a travelling oven. When cool, the bread was sliced into $\frac{1}{2}$, $\frac{7}{8}$, and $1\frac{1}{4}$ inch slices,

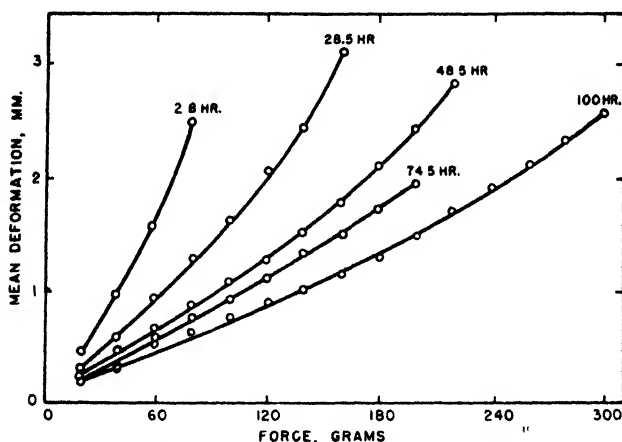


Fig. 9. Force-deformation curves for commercial white bread. Force vs. mean deformation on $1\frac{1}{4}$ " slices.

cut into squares $2\frac{3}{4} \times 2\frac{3}{4}$ inches and then placed in random fashion in air-tight cans at room temperature.

The first readings were obtained at approximately two hours after removal from the oven, and subsequent determinations were made at 24-hour intervals up to and including 100 hours. Fifteen slices were measured for each slice thickness, at each storage period. On each slice a series of deformation readings were obtained at successive 20-gram increments. The curves obtained with slices $1\frac{1}{4}$ inch thick shown in Fig. 9 are typical of the series. In all cases there was a curvilinear relation between load and deformation particularly for the freshest breads. The standard errors for comparable deformation values were considerably lower than those obtained with canned bread but they were still very high; the standard errors of single determina-

tions expressed in per cent of the mean varied from 11.0 to 33.9% for the one-half inch slices. As the slice thickness was increased the per cent error also increased and the greater area of the compression plate in this series did not reduce the error to desirable levels. With laboratory baked bread, the force-deformation relations were more curvilinear at corresponding staling times than were found for the commercial bread. It therefore appears that if the deformation exceeds a very low value, the force-deformation ratios are not constant.

It should be emphasized that the preceding force-deformation data were all obtained by reading the deformation obtained under standard force increments and that the error involved in attempting to read variable deformation is large.

The error is markedly lower, however, when force-deformation data are obtained by reading force (instead of deformation) required to give a series of arbitrary deformation values. By this method, the standard errors of a single determination (expressed as a per cent of the mean) have been found to be in the order of 9 to 10% for 2.5 mm. deformations.

Apparently, one can read variable force values with a considerably greater degree of accuracy than when attempting to estimate the small changes on the deformation scale of the Baker compressimeter. This fact represents another valid reason for determining "firmness" rather than "softness" when evaluating staling with the Baker compressimeter.

The force-deformation data obtained by reading variable force values, however, likewise yield curvilinear relationships, exemplified in Fig. 10.

The question naturally arises as to whether bread obeys Hooke's law under the conditions employed. That is, whether strain is proportioned to the stress. Since strain equals the deformation divided by the original crumb thickness (a constant), it is not necessary to transform the deformation readings in order to test the relationship. However, stress equals the force divided by the effective cross sectional area of the test object and since this effective area of force changes during deformation, it would be desirable to calculate the stress values.

In the present study, crumb slices slightly larger than the area of the plunger were used, and it was apparent from observation of the bending of the crumb surrounding the plunger, that the effective area under load was increasing with increments of force.⁶ Dividing the force readings of Figs. 8, 9, 10 by increasing area values in order to obtain stress readings would yield stress-strain curves which would

⁶ The change in cross-sectional area under compression represents another reason for employing a "firmness" method for evaluating staling changes, since all comparisons are made on bread at the same standard compression and hence same effective cross-sectional area.

tend to be more curvilinear. Consequently, without attempting to evaluate the magnitude of the increase in effective area, it can be stated that the bread did not strictly obey Hooke's law.

It must be concluded that, under the conditions of these experiments, bread crumb does not behave as a perfectly elastic body but as a plasto-elastic solid. In the case of fresh crumb, plastic flow is an appreciable factor in the compression which is observed, but as the bread stales and becomes rigid, presumably by the establishment of a three-dimensional structure through the formation of cross linkages, the possibilities for plastic flow become more and more restricted and the behavior of the bread closely approaches that of a pure elastic body. It would therefore be expected that the elastic regain of stale

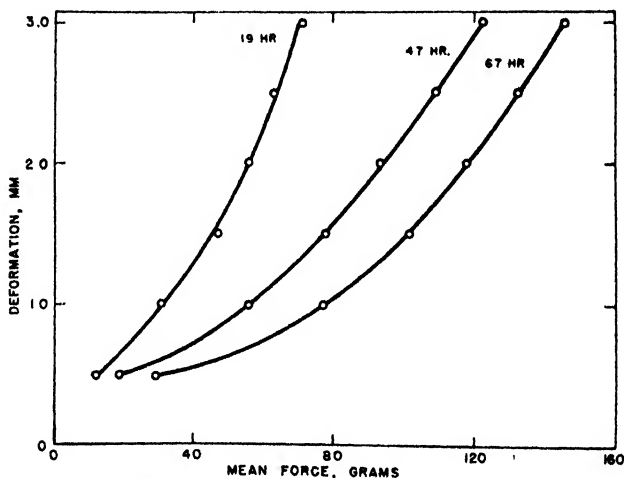


Fig. 10. Mean force values for laboratory baked white bread plotted against deformation for various staling times. Each point is a mean of 10 individual determinations.

bread would be greater than that of fresh bread; moreover, any treatment which would increase the softness would tend to cause the bread to deviate more from Hooke's law and, by increasing the possibility for plastic flow, would yield bread exhibiting poor elastic recovery from the application of stress.

While the failure of bread strictly to follow Hooke's law is not surprising because of its complex nature, it is a complicating factor in the use of the compressibility technique for following the changes in crumb hardness. The apparent rate of change will be influenced by the experimental conditions which are employed, such as the slice thickness and the standard deformation which is selected for firmness readings. Moreover, the results cannot be subjected to precise

mathematical treatment; the reciprocals of crumb softness data do not correspond exactly with crumb "firmness" readings, and the results cannot be expressed in absolute units.

Farinograph Method

The mathematical difficulties involved in attempting to evaluate the curvilinear Farinograph data have been pointed out in previous sections. In some instances these data may be converted to linear data, by appropriate mathematical manipulation; but there still remains considerable doubt as to what relation, if any, exists between the Farinograph data and bread staling. Several laboratory experiments have served to strengthen this doubt as may be seen from the following examples of studies on 40% starch pastes.

Maximum Farinograph consistency values obtained with 40% wheat starch pastes during storage decrease with time just as in the case of bread crumb. On the other hand, consistency values for 40% waxy starch pastes (corn and sorghum) increase with time, as though becoming "fresher," while over the same time interval these initially soft pastes set to form hard, almost cartilaginous gels. Typical results follow:

Age of paste	Maximum Farinograph consistency	
	40% wheat starch paste	40% waxy sorghum starch paste
<i>hr</i>	<i>B.U.</i>	<i>B.U.</i>
4	360	325
24	360	425
48	345	440
70	260	450
95	122	—

These results indicate that the Farinograph and "firmness" methods may give widely different results. In addition, it could be inferred that the physical changes in bread may involve more than changes solely in the amylopectin fraction of starch.

When polyoxyethylene stearate was added to a 40% wheat starch slurry in an amount equivalent to 0.5% of the total solids and the mixture gelatinized, the fresh paste was whiter, less sticky, and more crumbly than the control. Upon mixing the treated paste in the Farinograph, a coherent mixture was not formed; the particles merely rode on the blades without offering appreciable resistance to the mixing force with the result that a low or "stale" consistency reading was obtained which was not representative of the true consistency nor the age of the paste. After storing the treated paste for several weeks, it was still white and soft to the touch.

Similar anomalous results were obtained by Freilich (12) upon comparing the Farinograph and compressibility methods on bread containing this adjunct.

Measurement of Swelling Power

During staling, the ability of bread crumb to absorb water decreases. This so-called "swelling-power" change has been followed in the laboratory by several procedures in which crumb is first pulverized in water and the swelling power then determined by measurement of the crumb volume after sedimentation (16) or centrifuging (7); or by the increase in weight of crumb sediment after centrifugation of the slurry (22). Measurement of capillary flow of a thin pulverized crumb slurry has also been used (15).

Here again with this method, one apparently must exercise care in interpretation of results, as it was found that swelling power values for 40% waxy sorghum starch pastes *increased* with time instead of decreasing. Just as in the case of the Farinograph consistency values, such an increase would give the false impression that the paste was becoming "fresher," while over the same time interval, the stored pastes became very firm. Typical results are summarized below:

Age of paste hr.	Swelling power ¹	
	40% wheat starch paste	40% waxy sorghum starch paste
2	4.77	2.87
24	4.09	2.99
48	4.06	3.24

¹ Swelling power = $\frac{\text{weight centrifuged crumb sediment}}{\text{original weight crumb sample (dry basis)}}$
All values are means of duplicate determinations.

In applying such sedimentation methods to staling studies careful consideration must be given to the various factors which may be involved. For example, in applying this method to a study of the effects of chemical adjuncts, the sedimentation height of the crumb particles will depend upon their sedimentation velocity which in turn is influenced by several variables embodied in Stokes' Law of falling bodies.⁷ The adjunct might be adsorbed on the crumb particles and influence their specific gravity or crumb particle radius, or change the

⁷ Stokes' Law of falling bodies:

$$V = \frac{2}{9} \frac{(D - d)}{\eta} G r^2,$$

where V = velocity of fall;

D = the specific gravity of the falling particle;

d = the specific gravity of the medium through which the fall takes place;

η = the viscosity of the medium;

G = the gravity constant;

r = the radius of the particle.

viscosity of the dispersion medium. Hence it is difficult to interpret crumb sedimentation data, particularly when obtained on breads made by different formulas.

Measurement of Soluble Starch

The amount of soluble starch that can be leached from the crumb with water decreases with aging of the loaf. This method (16, 22) consists of precipitation by alcohol, recovery, and drying of this starch, but is so time consuming that it is not often used. Here again, one must be cautious in drawing conclusions when comparing treated with control breads. If, for example, an anti-staling agent were able to form a water-insoluble complex with the soluble starch, a low, or "stale" soluble-starch value would be obtained since the usual amount of soluble starch would not be extracted from the crumb. However, this could actually mean in this case that staling is being partially inhibited because the soluble starch would no longer be free to aid in building up an immobile three-dimensional gel structure.

Reference has already been made to the fact that Katz (16) found that the swelling power and soluble starch methods gave widely different results when used to evaluate the effect of acetaldehyde on bread staling.

Measurement of Crumbliness

The crumbliness values increase with time and would appear to be of interest in relation to consumer acceptance. It seems probable that a certain level of crumbliness would be associated with tenderness and ease of mastication whereas excessive crumbliness would be undesirable since the bread would not slice well. On the basis of this concept, a crumbliness method would have to be "calibrated" by consumer acceptance tests to determine the optimum crumbliness range before one would be in a position to state whether a change in crumbliness due to different formulas or treatments is desirable or undesirable. This reasoning also applies, of course, to other methods which have been used as indices of staling. This method is of somewhat limited value because of the difficulty in obtaining reproducible results.

Discussion

These studies serve to indicate the difficulties which are involved in following the staling of bread crumb by the laboratory methods which are in common use. Some of the contrary conclusions in the literature are attributable to differences in defining and measuring staling.

Staling, as it is applied to bread, is a generic term covering a number of ill-defined changes that occur in bread as it ages. Consumers judge

staleness by direct perception, which provides a subjective estimate that probably represents an unconscious integration of many factors. As bread ages certain phenomena are readily apparent: the flavor and aroma change (probably through loss of volatile constituents), the crumb becomes firmer, crumbles more readily and feels harsher to the tongue. These gross changes are doubtless the result of complex physico-chemical reactions within the loaf. These gross changes and the various underlying reactions, as well as other physical or chemical phenomena which contribute to the subjective estimate comprise the process commonly called "staling."

Investigators who are engaged in devising methods for measuring certain properties of bread and in determining how these properties change as bread ages tend to assume that each method measures "the rate of staling" and to argue the relative merits of different methods from this viewpoint. It seems more reasonable to suppose that any one physical or chemical method can measure, at best, only a few of the factors involved in the subjective integrated assessment of staling. Each method can reveal only part of the story. Moreover, since there is no *a priori* reason for supposing that the various processes involved in staling take place at the same rate, there is no reason to expect that methods that measure different properties will give identical data for rates of change in the crumb.

It appears that the comparative merits of various objective measures of bread staling can only be determined adequately through the use of organoleptic tests made by a panel of experts under a strict statistical control. The panel would hardly be able to make quantitative estimates of staleness, that is, to judge whether one sample was two or three times as stale as another; but given a series of bread samples, it should be possible to rank them at least roughly in order of "staleness." The physical or chemical measurements that place the same series in most nearly the same rank order as the panel may then be said to be the best objective method of measuring staleness.

Until a study of this nature is made, it seems that methods now in use must be accepted for what they actually are, namely, methods of measuring the change in some property or properties of bread with time. That some properties, such as crumb firmness and crumbliness, are related to staling is a reasonable inference but to suppose that any one method actually served as a measure of the whole process seems unjustified on the basis of our present knowledge.

Acknowledgments

The authors are indebted to P. P. Noznick who developed the modified empirical crumbliness test and to R. Koch and R. Stenberg for technical assistance in carrying out the comparative study of various methods for following the staling process. They are also grateful for the helpful suggestions of J. A. Anderson, Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, in preparing the manuscript.

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SOME PHYSICAL VARIABLES AFFECTING THE GASEOUS BLEACHING OF FLOUR ¹

W. W. DODGE and MAX MILNER

ABSTRACT

A number of physical variables, including time of gaseous treatment, agitator loading, moisture content of the flour, temperature, and time of reaction, influence the efficiency of flour bleaching with nitrogen trichloride, as determined from the carotene color value of flours treated in a laboratory-scale bleacher.

Bleaching efficiency decreased with extent of treatment, and pigment removal beyond 0.65 ppm. could not be obtained even at extremely high levels of treatment with the flour used in this study. Nitrogen trichloride reacted with flour at a very rapid rate. Duration of gas treatment, irrespective of amount of gas, was a major variable affecting efficiency, and this factor in turn depended on agitator loading. The mixing of bleached and unbleached flour conferred no bleaching on the untreated flour.

Temperature was inversely related to bleaching efficiency, indicating the possibility that adsorption, which is influenced negatively by temperature, limits the chemical action of nitrogen trichloride on flour pigments. At the same rate of application more pigment was destroyed at low temperatures; at higher temperatures more of the gas apparently reacted with other flour constituents. Increased moisture content of flour similarly caused a decrease in bleaching efficiency.

A simple test of efficiency of commercial bleaching equipment, in terms of pigment removal obtainable with a laboratory bleacher, is outlined.

The influence of a number of physical variables which may affect the commercial flour bleaching process was investigated with the objective of originating technics to secure optimum efficiency with gases which are now widely used for both bleaching and maturing. These factors, which included duration of gas application, temperature of flour, flour moisture content, agitator loading, and velocity of reaction, were studied with laboratory-scale equipment. The bleaching gas used was nitrogen trichloride. That some of these variables may have a major influence on bleaching efficiency was apparently overlooked in previous studies of gaseous flour bleaching which have stressed either the purely chemical aspects of the subject (2, 8, 9) or the comparative efficiency of various bleaching and maturing gases when applied under pilot-scale and standard mill conditions (3, 4, 5, 6, 7).

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A portion of a thesis presented by W. W. Dodge as partial fulfillment of the requirements for the degree of Master of Science in Milling Industry at Kansas State College.

Materials and Methods

The major portion of this study involved the use of a motor-driven laboratory flour agitator similar in design to that manufactured by the Wallace and Tiernan Company except that it was octagonal in shape and contained a system of deflecting paddles intended to increase the mixing efficiency. The agitator was maintained in a thermostatically controlled constant temperature cabinet and was driven at 29 rpm. Nitrogen trichloride was generated by forcing a measured quantity of chlorine gas into a solution of ammonium chloride contained in a reaction vessel. The nitrogen trichloride generated by the interaction of these reagents is aerated from the mixture into the flour agitator. The bleaching efficiency expressed as percentage of pigment removal was determined by the water-saturated N-butyl alcohol extraction method for flour pigments as outlined in *Cereal Laboratory Methods* (1), using an Evelyn photoelectric colorimeter. This determination was carried out 24 hours after the flours were bleached. The response of the standard flour to nitrogen trichloride treatment and the optimum treatment level were determined by a baking test carried out by the straight dough pup-loaf method described in *Cereal Laboratory Methods*, using 6% sugar, 4% milk, 1.5% salt, and 3% shortening, on a 14% moisture basis for the flour.

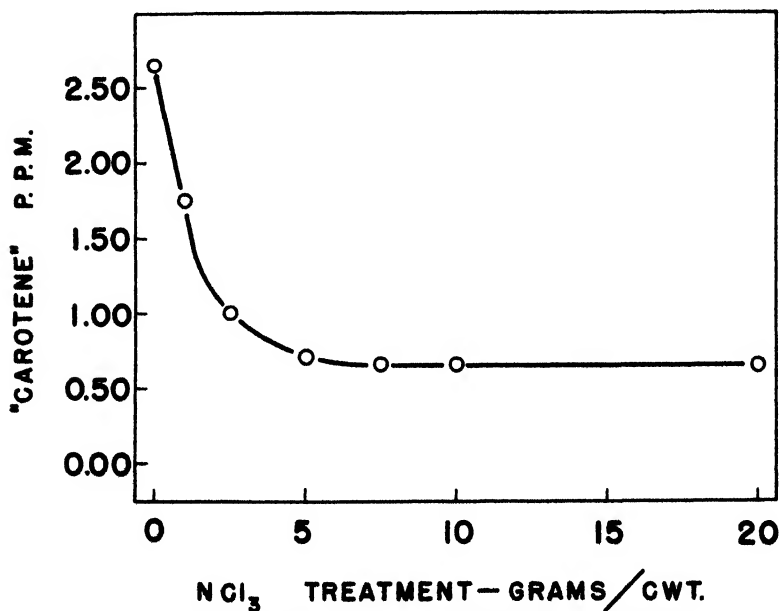


Fig. 1. Influence of dosage of reagent on pigment concentration.

The flour used throughout this study was a straight grade milled to 68% extraction from a typical commercial mill mix of southwestern hard red winter wheats, and was representative of the quality of the 1948 crop. It contained 11.7% protein, 14% moisture basis.

Results

Influence of Dosage on Extent of Bleaching. The influence of the dosage of nitrogen trichloride on bleaching efficiency at room temperature in terms of removal of pigment was investigated by analyzing for carotene flour samples treated at the following dosages (g./cwt.): 1.0, 2.5, 5.0, 7.5, 10.0, and 20.0. The data obtained in this experiment relating residual pigment concentration to bleaching rate are shown in Fig. 1. Reference to Fig. 1 indicates that the pigment removal from this flour is very great with treatments up to 2.5 g. of nitrogen trichloride per hundredweight, but is less rapid as the treatment is increased to levels as high as 7.5 g./cwt. No removal of pigment from this flour beyond 0.65 ppm. could be obtained with treatments higher than 7.5 g. This curve probably represents a normal pattern for all flours bleached with nitrogen trichloride and indicates that a residual pigment remains which is impervious to oxidation by this reagent. Normally, further bleaching is accomplished in mills by the use of benzoyl peroxide.

Influence of Duration of Gas Treatment and Agitator Load. The relationship between the time of addition of a fixed charge of gas to bleaching efficiency and the influence of variable loading on the time of gas application required for optimum efficiency was investigated. Nitrogen trichloride was introduced in the amount of 2.5 g./cwt. of flour in a bleaching program in which 2-, 4-, and 6-pound samples were treated over intervals from 25 seconds to 6 minutes as follows:

2-lb. samples		4-lb. samples		6-lb. samples	
Min.	Sec.	Min.	Sec.	Min.	Sec.
0	25	1	17	2	0
0	35	1	45	2	45
0	45	2	6	3	0
2	9	3	6	3	34
2	30	3	40	4	0
3	0	4	25	5	25
4	0	5	0	6	0

The 2.5 g. level of treatment was selected by a previous baking trial as the one which gave nearly optimum color removal with maximum improvement of baking properties. The results presented in Fig. 2 indicate that at light loading (2 lb.) maximum bleaching efficiency (61% pigment removal) could be obtained when the gas was introduced over an interval of 45 seconds, whereas with shorter time intervals

of gas introduction (25 and 35 seconds) a drastic decrease in bleaching efficiency occurred. Extension of the time of gas application beyond 45 seconds in this case caused no further increase in efficiency. A similar trend was noted with heavier loading of the agitator. The 4-lb. loading reached a maximum degree of color removal only after 2 minutes and 15 seconds of gas application, whereas the 6-lb. load required 4 minutes and 30 seconds. It is significant that the identical quantity of gas introduced over a short period did not produce as great

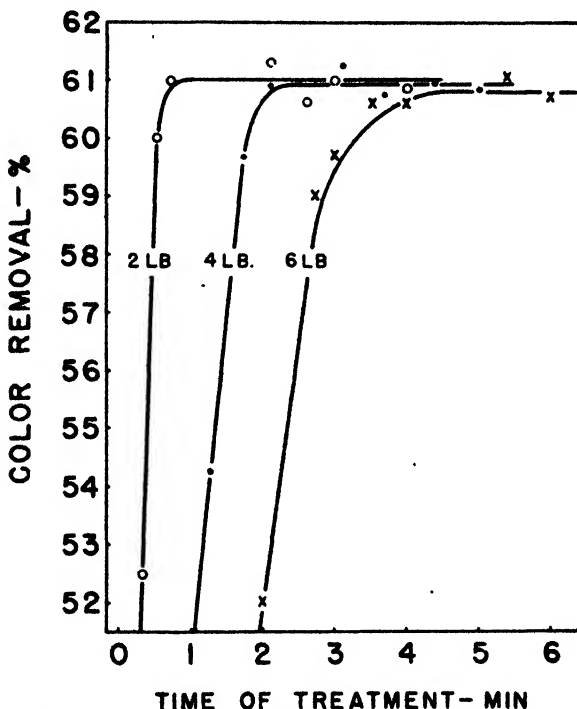


Fig. 2. Influence of time of treatment with nitrogen trichloride and agitator loading on color removal.

color removal as could be realized when the time of treatment was extended. These data indicate that a decrease rather than an increase in efficiency is obtained when the agitator load is increased with respect to time of gas treatment.

Estimate of Velocity of Reaction of Nitrogen Trichloride with Flour. To obtain an indication of the rate of reaction of nitrogen trichloride, 150 g. of flour were packed into a cylindrical glass tube between glass-wool plugs and perforated rubber stoppers as shown in Fig. 3. A quantity of nitrogen trichloride mixed with air to a volume of 2400 ml., sufficient to treat 150 g. of flour at the rate of 60 g./cwt., was forced

through the column of flour for 10 minutes at a pressure of 10 lb. per square inch.

Two distinct zones of color appeared in the tube of flour. A first zone of flour, approximately $\frac{1}{4}$ inch in thickness, appeared to be very white and showed a pink coloration at the first surface of contact of the gas with the flour. The balance of the flour in the column, however, appeared to be unbleached and the boundary between the bleached and unbleached flour was clearly defined. These relationships were confirmed by careful separation of the flour in the different zones and analysis for pigment content. The bleached zone, $\frac{1}{4}$ inch thick which contained 10% of the total flour in the tube, apparently had retained 100% of the bleaching gas. In spite of this high retention of nitrogen trichloride this bleached flour showed only a 60% removal of pigments, which is comparable to that obtained with normal commercial treatment (ca. 2 g./cwt.). The extreme rapidity of the action

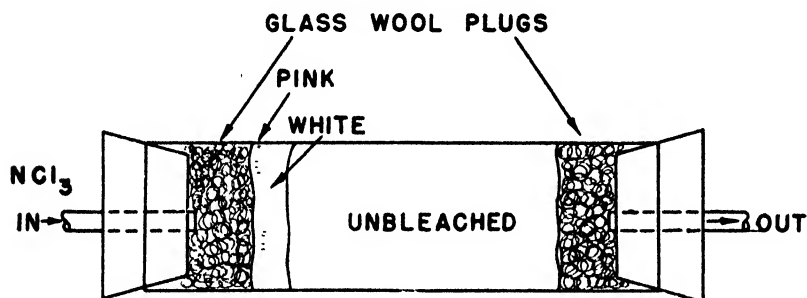


Fig. 3. Diagrammatic view of flour column in glass tube after treatment with nitrogen trichloride.

of nitrogen trichloride on flour is indicated by this experiment and the speed of the reaction can be calculated from the results.

Packed flour has about 52% of free air space, leaving 48% actually occupied by flour particles. Thus the cross-sectional area of the tube, which is 2.036 square inches, would be made up of $2.036 \times \frac{48}{100}$, or 0.975 square inches of flour, leaving 1.06 square inches of intergranular space for gas flow. The volume of gas used was 2400 ml. or 146.5 cubic inches and the time of passage was 10 minutes. Thus the rate of flow through the flour cross section was $\frac{146.5}{1.06 \times 10}$, or 13.8 inches per minute. Assuming that bleaching was complete in a zone 0.25 inch thick, the gas would pass through the bleached layer in only $\frac{13.8}{60 \times 0.25}$ or 0.92 second.

This calculation shows that any portion of the gas reacted with the first 10% of the flour column within one second from the time of initial contact.

Influence of Blending Bleached and Unbleached Flours. It has been shown that nitrogen trichloride reacts very rapidly with flour and that mixing efficiency decreases sharply with overloading. Under certain conditions it is possible, therefore, that the gas intended to treat a certain quantity of flour may react with only a portion of the total amount, with the result that part of the flour will remain either partially bleached or even entirely unbleached. Under practical conditions this could occur through the application of gas over too short a period

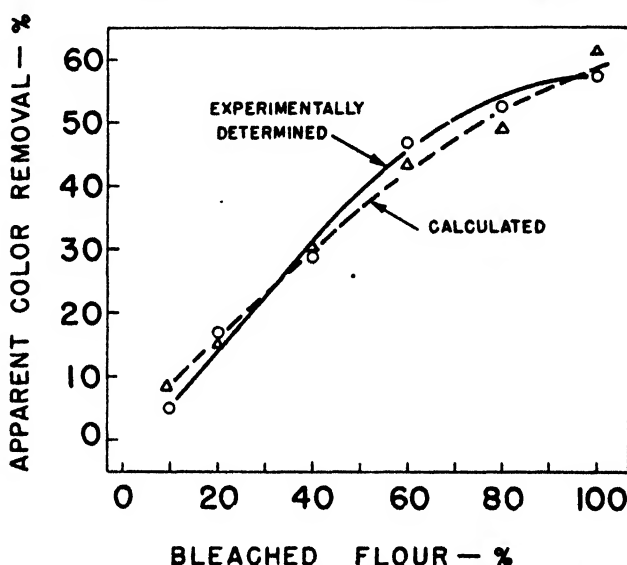


Fig. 4. Influence of blending bleached and unbleached flour on apparent color removal with nitrogen trichloride.

to effect uniform distribution of the bleaching gas, with the result that the total bleach is not equally distributed throughout all the particles of flour. It was the purpose of the following experiment to determine the influence of such conditions on bleaching efficiency.

Subsamples of a flour were treated with nitrogen trichloride to the following levels (g./cwt.): 25.00, 12.50, 6.25, 4.70, 3.13, and 2.50. Bleached lots were then blended with unbleached flour to yield samples all containing 2.5 g./cwt. of bleach and were analyzed for carotene by the usual method. The data obtained are presented in Fig. 4 together with the calculated values for the final pigment content, using the dilution factor of added flour. This curve followed that obtained experimentally very closely.

These results indicate clearly that overbleaching one portion of flour does not confer bleaching to unbleached flour mixed with it, and that the final pigment content is the weighted average of that of the two individual samples. In commercial practice one would probably be dealing with flour compounded of material underbleached to various degrees mixed with flour at various stages of overbleaching.

Effect of Temperature. To determine the effect of temperature on bleaching, the following experiment was carried out.

Identical 4-lb. samples of flour were sealed in glass bottles and brought to 7°C., 15°C., 26°C., 40°C., and 50°C. respectively in a controlled constant temperature cabinet. The various samples were

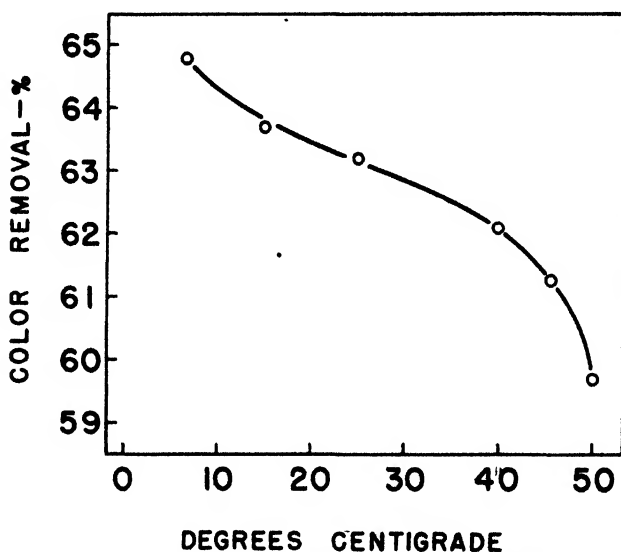


Fig. 5. Effect of temperature on bleaching efficiency with nitrogen trichloride.

bleached at these respective temperatures for 4 minutes at the standard rate of nitrogen-trichloride treatment (2.5 g./cwt.). The results, which are averages of duplicates, at each temperature are presented in Fig. 5.

The downward trend of the bleaching curve with increasing temperature was surprising since the rate of a purely chemical action can be expected to increase with an increase in temperature. Other explanations than purely chemical reaction phenomena must therefore be sought. Since adsorption has a negative temperature coefficient, it is highly probable that adsorption precedes chemical reaction in the bleaching of flour with nitrogen trichloride. It is to be noted, however, that regardless of the variation in efficiency of bleaching with tempera-

ture, the total amount of nitrogen trichloride applied to the flour was the same at all temperatures. This might indicate that as the rate of gaseous combination with flour is reduced by an increase in temperature, the less does the bleaching gas react with the pigment and the more do other flour components, particularly protein, enter into reaction with nitrogen trichloride. In other words, temperature, in determining the rate of adsorption, may affect the differential rate of reaction between nitrogen trichloride and various flour components.

The Effect of Temperature as Related to Time of Treatment. In the previous experiment, increasing temperature was found to decrease the

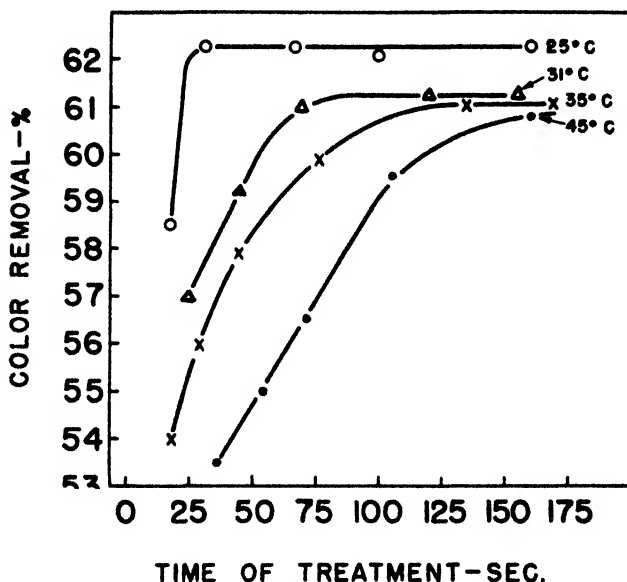


Fig. 6. Influence of temperature and time of treatment with nitrogen trichloride on color removal.

efficiency of bleaching when the gas was applied over a period of 4 minutes. It was of interest to ascertain whether increased efficiency at the higher temperatures might be obtained if the time of gas treatment were extended. For this experiment, flour was bleached over various time intervals at 25°C., 31°C., 35°C., and 45°C.

A representative experiment was as follows: Ten sealed bottles of flour, which had been maintained at 10°C., were placed in the constant temperature cabinet at 45°C. for about 16 hours before bleaching. Different intervals of treatment with nitrogen trichloride at the various temperatures were applied. All samples were bleached in duplicate and the average values are presented in Fig. 6.

At low temperatures, e.g., 25°C., maximum utilization of the gas is obtained in much shorter periods than at higher temperatures. As the temperature is increased, the time required to produce the maximum bleach is rapidly extended until at 45°C. the optimum bleach was reached only after 3 minutes, as compared to 0.5 minute at 25°C.

This experiment proves conclusively that temperature is inversely related to bleaching efficiency. As the temperature is increased, the

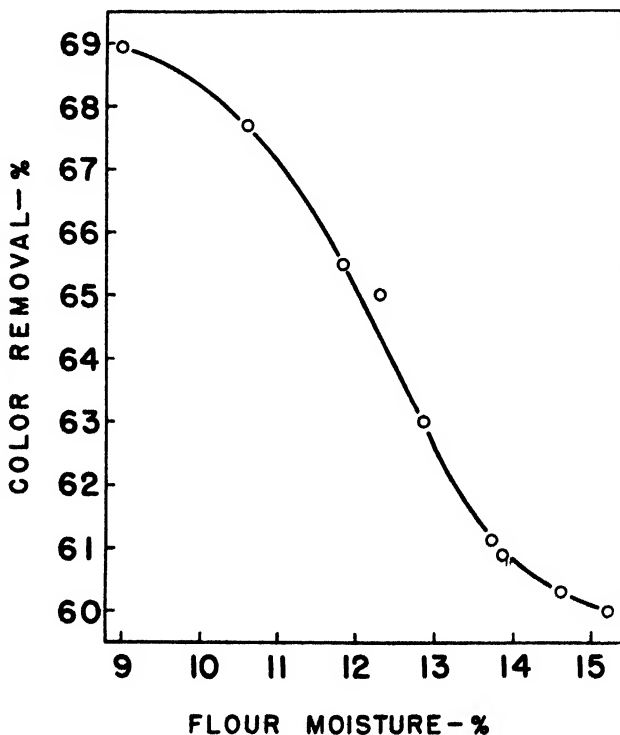


Fig. 7. Influence of flour moisture on color removal with nitrogen trichloride.

time required to obtain a maximum bleach is increased, while the efficiency with respect to color removal decreases as the temperature is increased.

Effect of Flour Moisture on Bleaching Efficiency. Moisture content is a major variable in flour processing, and any influence of this factor on bleaching efficiency with gases would be important to milling operations. A group of flour samples with moisture values ranging from 8.9 to 15.2% were bleached with nitrogen trichloride at the rate of 2.5 g./cwt. Bleaching time, temperature, and loading were held con-

stant in this experiment. Moisture content was determined by the air oven method as outlined in *Cereal Laboratory Methods*. The results of this study appear in Fig. 7.

These data do not bear out the widely held opinion in the milling industry that bleaching efficiency increases with moisture content, since exactly the reverse was found to be true. The present data indicate that a decrease in bleaching efficiency occurred throughout the moisture range investigated, namely 8.9 to 15.2%, and that the greatest downward inflection of the curve occurred in the region of 12% moisture. A possible explanation of this phenomenon is the low solubility of nitrogen trichloride in water. As the moisture content of

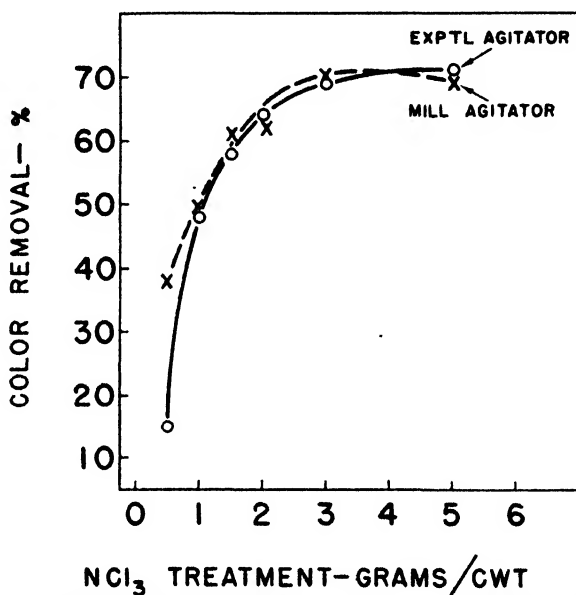


Fig. 8. Comparative efficiency of experimental agitator and commercial mill agitator.

a flour increases it may be expected that its affinity for nitrogen trichloride would decrease.

Relative Efficiency of Experimental Agitator and Commercial Scale Mill Agitator. Ferrari and co-workers (5) stated that pilot-scale bleaching is less efficient than bleaching on an experimental basis, and that the commercial bleaching process was even less efficient than that carried out on pilot scale. It was of interest, therefore, to compare the efficiency of the small box agitator with the commercial No. 1 Alsop agitator in the Kansas State College mill. The latter was operated at 135 rpm. and a load of 500 lb. per hour, which is the normal production rate of the mill and is very low for agitator equipment of this

size. Using commercial Agene generating and metering equipment, samples were bleached at the following rates (g./cwt.): 0.5, 1.0, 1.5, 2.0, 3.0, and 5.0. An unbleached lot of this flour was taken for treatment in the experimental agitator at the same rates using a loading of 2 lb. and a time of gas treatment of 4 minutes. The percentage of color removal obtained in both studies is given in Fig. 8.

These curves show that no significant difference existed in the efficiency of bleaching between the mill and experimental agitator. This result differs from those obtained by Ferrari and co-workers (5) who found the experimental type to be more efficient. The high efficiency of the mill bleacher in the present study was due to the fact that it was very lightly loaded. In any event it is apparent that commercial equipment can be operated to yield optimum utilization of bleaching gas and that many of the variables affecting its efficiency still remain to be determined.

Discussion

These experiments indicate that simple physical variables which normally may be encountered in flour bleaching with gases may profoundly affect the efficiency of the process. The very rapid rate of reaction of nitrogen trichloride with flour must be taken into account in determining the time to be used for introducing a given amount of gas, in order to obtain optimum bleaching efficiency. The inverse relationship of bleaching efficiency to temperature and moisture content shown in the present studies has not been generally recognized and is of fundamental importance in any consideration of methods to improve the bleaching process. The results of these studies have emphasized the complex nature of the reaction of nitrogen trichloride with flour. Pigments, fats, and proteins all appear to be affected by nitrogen trichloride, and physical variables such as temperature, moisture, extent of treatment, and duration of treatment will influence the degree of reaction of the various components of the flour with the gas.

A few facts regarding gaseous bleaching, which hitherto have been generally assumed, have been demonstrated experimentally in this study. These include the fact that the mixing of bleached with unbleached flour confers no bleaching to the untreated portion. It also has been clearly shown that nitrogen trichloride treatment allows a residual pigment, which is unaffected by enormous concentrations of the gas, to remain in the flour. It was further shown that an increase of gas concentration with increased agitator loading does not compensate for the loss in mixing efficiency caused by the increased load. Application of these laboratory-scale results may enable commercial bleaching to approach the laboratory batch process in efficiency.

It appears desirable to express the efficiency of commercial bleaching with gases by a numerical value which would take into account the pigment content of the original unbleached flour as well as the maximum bleach which can be applied to the flour by the most efficient methods. The maximum bleach for a given gas level would be that secured with an efficient laboratory agitator. This value in ppm. of carotene would be obtained by treating the unbleached flour with the gas at the same rate at which it was bleached in the commercial-scale agitator. This test bleach must be conducted at the same temperature and moisture content as in the commercial agitator.

The calculation of the empirical efficiency rating requires the assumption that the color value of the commercially bleached flour is an average of two lots of flour mixed together, one bleached to the maxi-

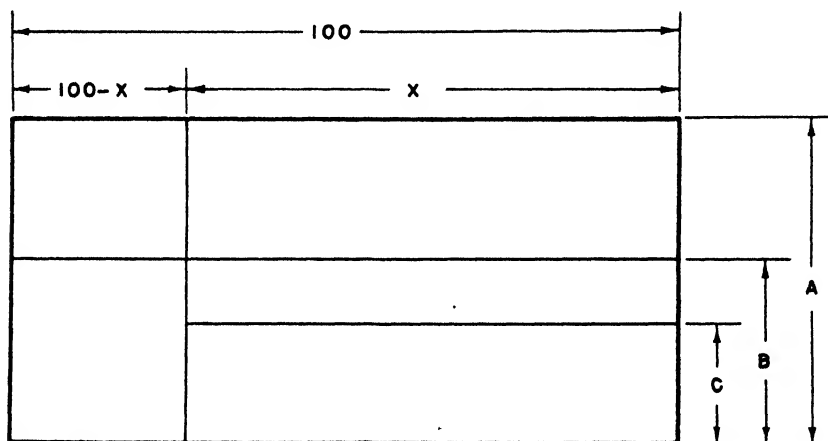


Fig. 9. Diagram for geometric development of equation for efficiency.

um value obtainable in the laboratory bleacher, and the other entirely unbleached. The calculation of efficiency therefore involves not only flour pigment content in terms of ppm. carotene, but also the relative quantities of the two flours (bleached and unbleached) in the mixture. Pigment determinations on unbleached flour, commercially bleached flour, and laboratory bleached flour are the only analytical values which need to be secured.

A geometric development of an equation for efficiency may be made with the aid of a diagram, as shown in Fig. 9.

Let A equal the color content in ppm. of the unbleached flour.

Let B equal the color content in ppm. of the commercially bleached flour.

Let C equal the color content in ppm. of the laboratory bleached flour. In this case A is greater than B which is greater than C.

X equals the portion of the commercially bleached flour, expressed as a percentage, which contains the maximum bleach.

100 - X equals the percentage of unbleached flour in the commercially bleached sample.

Area due to commercially bleached flour equals 100B.

This area may be equated to the sum of the areas CX + A (100 - X).

Equating these values:

$$100B = CX + A(100 - X).$$

Solving for X:

$$X = 100 \times \frac{(B - A)}{(C - A)}.$$

The term X can be visualized as that portion of the commercially bleached flour expressed in per cent which contains a bleach value equivalent to that obtainable with the laboratory bleacher and might well be called the agitator efficiency, or simply A.E.

$$\text{Thus} \quad \text{A.E.} = \frac{(B - A)}{(C - A)} \times 100.$$

As an example of the application of this equation, assume a practical case where the unbleached, commercially bleached, and laboratory bleached flours have color values of 2.5, 1.2, and 0.9 ppm. of carotene, respectively. The agitator efficiency, A.E., of the commercial equipment in the case of this example is

$$\frac{(1.2 - 2.5)}{(0.9 - 2.5)} \times 100 = 80\%.$$

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METHODS FOR THE LABORATORY-SCALE PRODUCTION OF CHLORINE DIOXIDE AND THE TREATMENT OF FLOUR¹

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ABSTRACT

Two practical methods for the small-scale generation of chlorine dioxide have been developed. In the first method, pure chlorine dioxide is measured volumetrically, followed by dilution with air and its subsequent application to the flour. In the second method, chlorine dioxide is prepared in aqueous solution (by the reaction of acetic anhydride with sodium chlorite); the gas is then aerated off and directly applied to flour.

Since chlorine dioxide has been approved as a flour maturing and bleaching agent, a safe, practical, simple, and reliable method for generating chlorine dioxide and for treating flour on a laboratory-scale is needed. Such a method should make most efficient use of existing laboratory equipment and facilities.

Hutchinson and Derby (3) have described three methods for the small-scale generation of chlorine dioxide, based on reacting chlorine gas with solid sodium chlorite. There are some handicaps in the practical use of these procedures. In one method, a 3-hour reaction period for the generation of chlorine dioxide gas is required. In other procedures described for noncontinuous and continuous production of chlorine dioxide, somewhat unreliable results were obtained or undesirable procedures were encountered. For example, for the preparation of chlorine dioxide by the use of chlorine gas and technical flake chlorite, considerable amounts of chlorine had to be passed through the chlorite tower before conditions stabilized. Some chlorine reacted with the soda ash contained in the chlorite and the yields of chlorine dioxide from a given amount of chlorine gas-air mixture passed through the tower could not be reproduced from day to day. Additional experiments showed that the moisture content of the air passed through the tower greatly altered the yield of chlorine dioxide. A continuous method for the generation of chlorine dioxide, described by these authors, required considerable amounts of equipment and numerous calibrations. Also, after the system is started, time is wasted until a constant output is reached. Titrations must be performed to check the rate of production before small samples of flour can be treated with some degree of control.

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Two practical methods for the small-scale generation of chlorine dioxide are described in this report.

Diluted Chlorine Dioxide Method

Materials and Methods. For earlier experiments, the Cunningham and Losch method (2), in which pure chlorine dioxide is generated by passing chlorine gas slowly through 25% sodium chlorite^a solution, was used. Satisfactory results were obtained with 100-ml. glass-stoppered gas washing bottles, preferably two in a series, connected with Tygon tubing, as illustrated in Fig. 1.

The bottles are nearly filled with chlorite solution but space is allowed for bubble formation to avoid any solution spilling over to the next bottle or out of the exit. Chlorine is led from a cylinder equipped

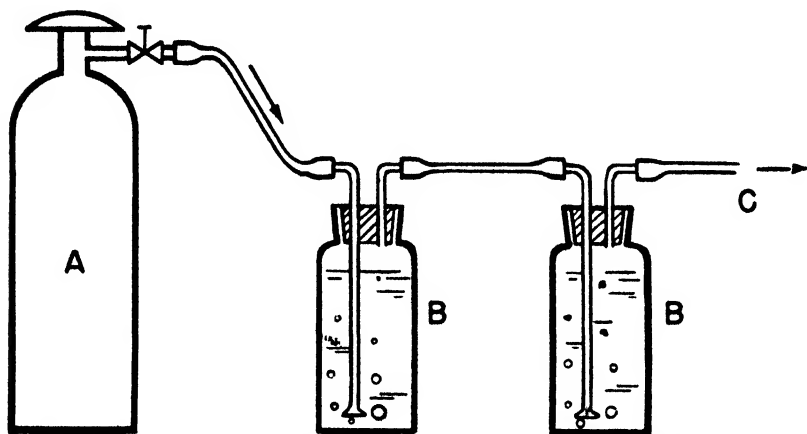


Fig. 1. Schematic diagram of apparatus for production of pure chlorine dioxide gas. A. Chlorine cylinder; B. Gas washing bottles containing 25% sodium chlorite solutions; and C. Pure chlorine dioxide gas exit.

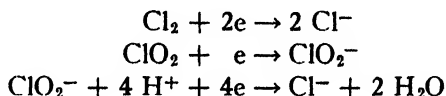
with a needle valve and bubbled through the chlorite solution. Three distinct reaction periods may be observed. At first, an induction period occurs when chlorine is absorbed by the solution and the chlorite solution turns dark. During this early period, the chlorine dioxide coming out of the exit is diluted with air. Second, a stable period is reached when nearly pure chlorine dioxide is delivered from the exit tube. During the third period, the chlorite solution turns from a dark brown to a straw color and chlorine gas begins to evolve.

Samples of pure chlorine dioxide gas are taken from the exit tube during the stable period by filling a pipette or gas burette with the gas. Flour is treated by diluting a calculated amount of pure chlorine

^a It is important to remember that sodium chlorite solution is very active. Also, the crystalline or flake sodium chlorite should be protected against contamination with organic matter, in order to avoid fires and explosions.

dioxide gas with air. The gas mixture is then passed through a mixing chamber and slowly applied to flour in an Agene Demonstrator Agitator. For varying treatments, a series of pipettes can be calibrated for treating 4 lb. of flour, or given volumes of gas may be taken and the flour weight varied.

For accurate control, samples of the gas should be analyzed by the method of Woodward, Petroe, and Vincent (5), which will be described later in more detail. A useful addition to their method is to buffer the potassium iodide solution to pH 8 with phosphate buffer. Free chlorine and one-fifth equivalent of chlorine dioxide reacts with potassium iodide under neutral and slightly alkaline conditions. Four-fifths equivalent of chlorine dioxide reacts with potassium iodide only under acid conditions. Electronically, this may be illustrated as follows:



If the potassium iodide should be slightly acid, some of the four-fifths equivalent of chlorine dioxide may liberate iodine slowly which will titrate as apparent chlorine. Therefore, buffering of the potassium iodide solution is a more accurate procedure. If the titration of iodine liberated at pH 8 is one-fifth of the total titration under acid conditions, pure chlorine dioxide is indicated. If the titration at pH 8 is more than one-fifth of the total titration under acid conditions, the gas is contaminated with chlorine.

Examples of typical titrations of equal volumes of gas taken from the generator and illustrating the induction period, and also the period after stability was reached, are listed in Table I.

TABLE I
ILLUSTRATING INDUCTION PERIOD IN GENERATING CHLORINE DIOXIDE BY
PASSING CHLORINE GAS THROUGH SODIUM CHLORITE SOLUTION
(Analyses of successive 5.5 ml. gas samples)

Gas sample	Titer of iodine liberated by ClO_2 with 0.1 N sodium thiosulfate	
	pH 8	Acid conditions
No.	ml.	ml.
1	1.9	9.0
2	2.1	10.1
3	2.1	10.5
4	2.1	10.5

The gas samples were analyzed consecutively at 10-minute intervals.

Table II lists gas analysis values after the generator had reached stable conditions and after excess chlorine had started to evolve.

TABLE II
ANALYSIS OF CHLORINE DIOXIDE GAS GENERATED BY PASSING
CHLORINE THROUGH SODIUM CHLORITE SOLUTION

Gas sample No.	Volume of gas sample at 23°C.	Titer of iodine liberated by ClO_2 with 0.1 N sodium thiosulfate		Volume of chlorine dioxide found	Volume of chlorine found
		pH 8	Acid conditions		
	ml.	ml.	ml.	ml.	ml.
1	25	10.5	50.1	24.0	0.7
2	25	10.3	50.1	24.1	0.5
3	25	10.5	50.4	24.2	0.6
4	25	11.2	48.7	22.3	2.2

Gas sample No. 4 illustrates the break-through period since appreciable amounts of chlorine were found.

Advantages and Disadvantages of This Method. The advantages of this method are the relatively simple setup for the generator and the excellent control obtained when the gas samples are taken after conditions have stabilized.

Among the disadvantages are: (1) the hazards of dealing with nearly pure chlorine dioxide gas; (2) the method is wasteful in respect to the use of chlorite, especially if only one or two samples of flour are treated; (3) the procedure is somewhat cumbersome because it requires an equilibrium condition dependent on time, and needs constant checking of the purity of the gas delivered, if precision is desired.

Caution and Comments. All glassware must be absolutely clean. Rubber stoppers and rubber tubing cannot be used. A hood or other method of disposal of the excess gas must be provided. A partially exhausted chlorite solution should be disposed of *at once* because it is saturated with chlorine dioxide gas which comes out of solution on standing and may then cause explosions.

For mill control work, gas analysis determinations may not be necessary.

Acetic Anhydride Method

A more desirable method for generating chlorine dioxide on a small scale would be to generate the gas in solution and then remove it by aeration. Aston⁴ (1) was granted a patent in February, 1948, on the production of chlorine dioxide by reacting organic anhydrides with aqueous solutions of chlorites. A laboratory method for generating chlorine dioxide and for bleaching flour on a small scale has been developed from this reaction. After extensive experimental work, a procedure has been evolved in which the yield of chlorine dioxide is

⁴ The use of patent No. 2,436,134 for laboratory purposes is permitted by Mathieson Chemical Corporation through private communication.

proportional to the chlorite used within the range for treating experimental batches of flour. The details of the procedure are as follows:

Reagents:

1. Sodium chlorite solution: 0.1% for 1-lb. treatment and 1% for 4-lb. treatment.
2. Sodium acetate buffer solution, 2 *M*: 164 g. of anhydrous sodium acetate are dissolved in distilled water and made to a volume of 1 liter.
3. Acetic anhydride, 95%.

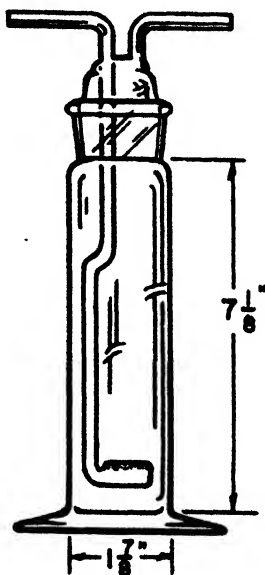


Fig. 2. Chlorine dioxide generator bottle comprising a pyrex gas washing bottle (Inter-joint, capacity 250 ml., stopper 40/35) with fused-in fritted disc (medium or coarse porosity), 20 mm. diameter.

4. Potassium iodide solution, 5%, buffered to pH 8 with phosphate buffer (95 g. disodium hydrogen phosphate and 5 g. potassium dihydrogen phosphate are made to a volume of 1 liter). 10 ml. of buffer mixture are required for each liter of 5% KI solution. If the pH is not 8 (± 0.1) it is adjusted with either the alkaline or acidic constituent of the buffer mixture.
5. Acetic acid solution, 50%.
6. Sodium thiosulfate solution, 0.1 *N*.
7. Starch indicator, 2% solution.

Apparatus:

1. Supply of compressed air or other sources of air, such as foot bellows or hand pressure bulbs.

2. Gas washing bottle with fritted glass diffuser of medium or coarse porosity and 20 mm. diameter; bottle capacity, 250 ml.; ground glass stopper. This bottle is to be used as the gas generator (Fig. 2).
3. Flour agitator such as supplied with the Novadel-Agene Demonstrator unit.

Procedure for the Preparation of a Calibration Curve:

1. The exact concentration of the sodium chlorite solution must be determined by reacting 5 ml. of 1% or 50 ml. of 0.1% aliquots with acidified potassium iodide and titrating the liberated iodine with 0.1 *N* sodium thiosulfate solution.
2. Fifty ml. of acetate buffer are placed in the chlorine dioxide generator, then chlorite solution is added. Finally, 1 ml. of acetic anhydride is added and the generator is closed and shaken vigorously for about 15 seconds. Immediately thereafter, the gas is aerated from the reaction mixture until the waste solution is colorless (about 15 minutes).
3. The gas must be analyzed quantitatively and qualitatively by passing it through two gas washing bottles containing 30 ml. of 5% potassium iodide solution buffered to pH 8. The liberated

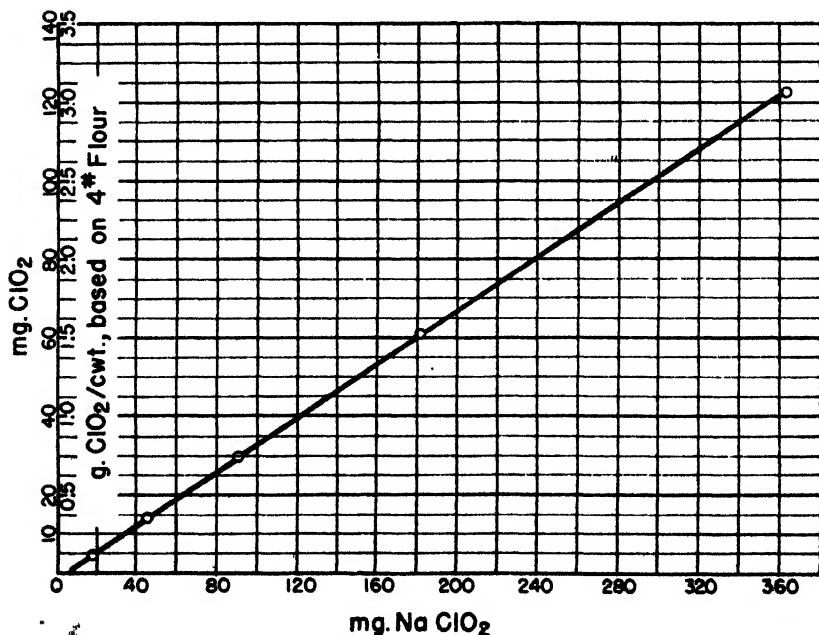


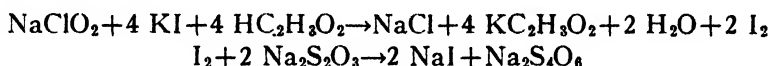
Fig. 3. Calibration curve for treating 4 lb. flour by the acetic anhydride method.

iodine is first titrated at pH 8 with 0.1 *N* sodium thiosulfate in the presence of 1 ml. of starch indicator. A second titration is performed after the same solution has been acidified with 25 ml. of 50% acetic acid solution.

4. Several determinations of chlorine dioxide yields should be made by using different quantities of sodium chlorite solution in order to prepare a calibration curve as shown in Fig. 3.

If the sodium chlorite solution is stored in a brown-colored bottle and out of direct sunlight, it will remain stable for several weeks or longer.

For verification of the sodium chlorite content of solutions: 5 ml. aliquots of 1% chlorite solution, or 50 ml. of 0.1% solution, are mixed with 1 g. of potassium iodide. When the crystals are dissolved, 20 ml. of 50% acetic acid solution are added and the mixture is allowed to stand 5 minutes in a dark place before titration with 0.1 *N* sodium thiosulfate solution.

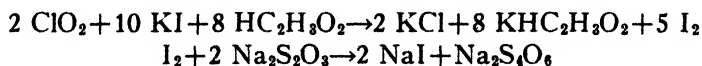


Calculations:

Grams of sodium chlorite contained in aliquot = ml. of standard thiosulfate \times normality \times molecular weight of $\text{NaClO}_2/4000$.

For checking the weight of chlorine dioxide produced, the method outlined in point (3) under "Procedure for preparing a calibration curve" is used.

The reaction is presented by the following equations:



Calculations:

Grams of chlorine dioxide titrated = 5/4 of acid titration with thiosulfate \times normality \times molecular weight of $\text{ClO}_2/5000$.

In Table III are illustrated the amounts of chlorine dioxide produced with increasing amounts of sodium chlorite when maintaining the foregoing conditions.

To obtain a measure of the reproducibility of the amounts of chlorine dioxide generated, four additional determinations were made with the different amounts of sodium chlorite listed in Table III and the results are summarized in Table IV.

The average values have been employed in the calibration curve shown in Fig. 3 for the treatment of 4 lb. of flour.

The amount of chlorine dioxide treatment per 100 lb. of flour when 4 lb. of flour are used in the experimental agitator can be read on the vertical axis.

TABLE III

GENERATION OF CHLORINE DIOXIDE FROM SODIUM CHLORITE BY REACTION WITH ACETIC ANHYDRIDE; CHLORINE DIOXIDE PRODUCED FROM VARYING AMOUNTS OF SODIUM CHLORITE

NaClO ₂	Titer of iodine liberated by ClO ₂ with 0.1 N sodium thiosulfate		ClO ₂ generated
	pH 8	Acid conditions	
<i>mg.</i>	<i>ml.</i>	<i>ml.</i>	<i>mg.</i>
18.1	1.0	3.8	4.7
45.3	2.6	11.4	14.9
90.6	4.8	22.9	30.6
181.2	9.9	47.2	62.2
362.4	20.9	97.5	129.3

Numerous cereal laboratories mill 1000-g. samples of wheat experimentally and about 500 to 700 g. of flour are obtained from each test. To treat such small quantities of flour, the calibration curve shown in Fig. 4 has been prepared for use with 1-lb. samples of flour. This curve is based on the data given in Table V. The conditions of generation were the same as previously described, except a 0.1% sodium chlorite solution was used.

Application of Gas to Flour. The component parts of the apparatus for treating flour by the acetic anhydride method are schematically represented in Fig. 5. If a source of compressed air is at hand, a needle valve is used for regulating the air flow through the system, but pressure bulbs with a reservoir or foot bellows can also be used. It is advisable to use a screw clamp in the air-supply line for regulating the air flow.

The chlorine dioxide needed to treat a 4-lb. sample or a 1-lb. sample of flour and the amounts of sodium chlorite needed for generating such quantities of gas may be read from the calibration curve. When treating flour, the chlorine dioxide gas diluted with air is passed into

TABLE IV

GENERATION OF CHLORINE DIOXIDE FROM SODIUM CHLORITE BY REACTION WITH ACETIC ANHYDRIDE

(Results of experimental trials made on five different days)

NaClO ₂	ClO ₂ generated					Mean
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	
<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
18.1	4.7	5.0	4.6	4.5	5.0	4.8
45.4	14.9	14.1	14.7	14.0	14.1	14.4
91.0	30.6	29.6	30.5	30.2	30.4	30.3
182.0	62.2	62.2	62.8	62.9	62.2	62.5
364.0	129.3	126.9	124.1	124.1	126.0	126.1

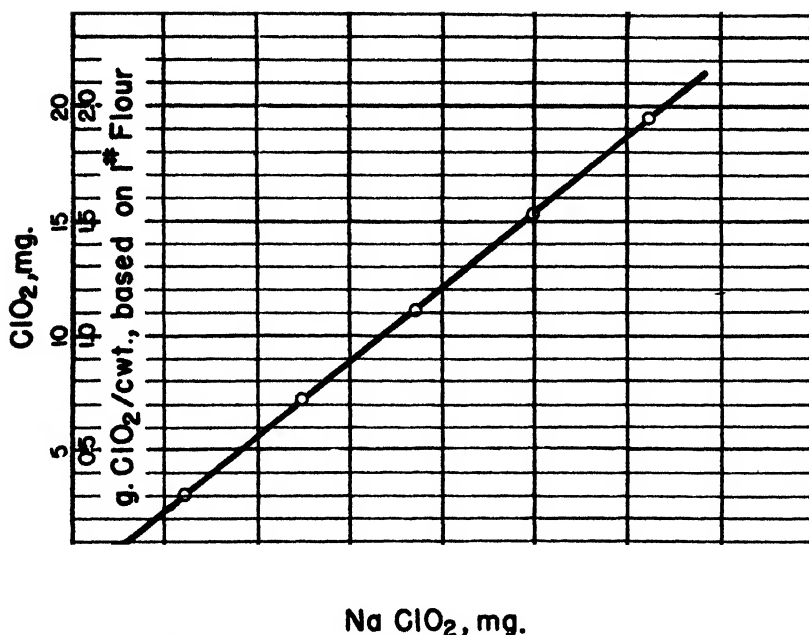


Fig. 4. Calibration curve for treating 1 lb. flour by the acetic anhydride method.

the flour agitator and aeration continued until the reaction mixture becomes colorless (10 to 15 minutes).

An air current of approximately 1 liter per minute is used for stripping the gas from solution. Less air will require a longer time, whereas more air will shorten the aeration time and there is danger of forcing chlorine dioxide out of the agitator before it has reacted with the flour.

Comments on Operation. The solution after aeration has a pH of 5.5 to 6.0 and has an iodine equivalent of 0.3 ml. 0.1 *N* sodium thiosul-

TABLE V
GENERATION OF CHLORINE DIOXIDE FROM SODIUM CHLORITE
BY REACTION WITH ACETIC ANHYDRIDE

(Data for treating 1-lb. samples of flour with chlorine dioxide)

NaClO ₂	ClO ₂ generated						Mean
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
5.0	0.7	0.8	0.8	0.7	0.7	0.7	0.7
12.4	3.1	3.1	3.2	3.0	3.1	3.1	3.1
24.9	7.1	7.2	7.3	7.3	7.1	7.1	7.2
37.2	11.0	11.1	11.0	11.0	11.5	11.1	11.1
49.8	15.2	15.5	15.2	14.8	15.6	15.3	15.3
62.3	19.6	19.5	19.7	19.3	19.6	19.5	19.5

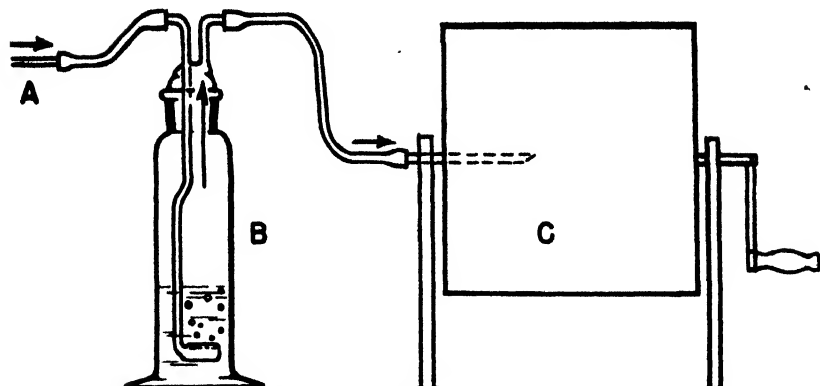


Fig. 5. Schematic diagram of apparatus for the laboratory treatment of flour by the acetic anhydride method. A. Air inlet (1 liter per min.); B. Generator bottle; and C. Flour agitator.

fate. A number of variables influenced the liberation of chlorine dioxide. When 100 ml. of 2 *M* acetate buffer were used instead of 50 ml. there was no appreciable alteration in the yield of chlorine dioxide. If less than 50 ml. of buffer solution were used, the waste solution became more acid, the yields were lower, and a longer period was required for stripping the gas from solution.

The addition of saturated sodium chloride solution to the reaction mixture increased the chlorine dioxide yield and decreased the aeration time somewhat; however, the resultant gas contained more apparent chlorine.

The use of less than the specified amount of acetic anhydride reduced the yield, but larger amounts served no useful purpose.

Glass or Tygon tubing should be used for conducting the gas from the generator to the agitator. Rubber stoppers, rubber tubing, or diffusers made of plastics are not recommended. Before using any new equipment it is important to saturate material which reacts with chlorine dioxide, in order that the flour be accurately treated.

The concentration of chlorine dioxide gas in the air emerging from the generating bottle was found to be very low, as shown by the

TABLE VI

CONCENTRATION OF CHLORINE DIOXIDE IN AIR WHILE TREATING
4-LB. FLOUR WITH 2 G. CHLORINE DIOXIDE PER CWT.

Time at which gas samples were taken	Chlorine dioxide (by volume) %
At once	1.2
After 5 minutes	0.8
After 10 minutes	0.1

typical analysis recorded in Table VI. This percentage of chlorine dioxide-in-air is far below the level where any explosion could possibly occur and compares favorably with commercial generation in the mill where 0.5% by volume is used.

Advantages and Disadvantages. The acetic anhydride method of generating chlorine dioxide was found to be safe and reliable. After a calibration curve has been prepared, small samples of flour can be treated in a very short time at any desired practical level. The reagents used for this purpose are quite stable and only an occasional titration is necessary for measuring the sodium chlorite content of the stock solution. There is no hazard due to the use and presence of compressed gases in the laboratory.

The chief disadvantage appears to be the use of an all-glass gas washing bottle with a fragile glass tubing holding the diffuser. Also, the comparatively long aeration period is a disadvantage in laboratories where a supply of compressed air is not available and when the flour agitator is turned by hand.

Comparison of Treatments with Pure Chlorine Dioxide and Gas Generated by the Acetic Anhydride Method

To show that both methods produced the same results, flours were treated at equal levels with chlorine dioxide. Color removal was measured by the Pekar test and by the extraction and determination of carotene, using water-saturated normal butyl alcohol.

TABLE VII

COMPARISON OF BLEACHING RESULTS OBTAINED WITH VARIOUS DOSAGES OF CHLORINE DIOXIDE GENERATED BY THE DILUTED CHLORINE DIOXIDE AND ACETIC ANHYDRIDE METHODS

Flour	Treatment ClO ₂ /cwt.	Carotene in flour	
		Dilution method	Acetic anhydride method
	g.	ppm.	ppm.
Patent	0.64	0.95	0.97
Clear, highly pigmented	2.86	1.25	1.23
Patent	0.25	1.57	1.52
Patent	0.69	0.98	0.94

The results recorded in Table VII show that there is no significant difference between the two methods. The dosages which were employed are not necessarily optimum for the best maturing action.

Further studies of the methods and also the relation between laboratory and mill treatments are being continued.

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THE DETERMINATION OF PERSULFATE IN FLOUR AND DOUGH¹

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ABSTRACT

A method has been developed for the quantitative determination of as little as 5 to 10 parts ammonium persulfate per million parts of flour or dough. The method involves the re-oxidation of leuco-fluorescein to fluorescein through the action of persulfate ion. Under controlled conditions, the intensity of the developed fluorescence bears a reproducible relationship to the persulfate content of the sample.

As part of an investigation designed to assess the utility of ammonium persulfate $[(\text{NH}_4)_2\text{S}_2\text{O}_8]$ as a flour maturing agent, it seemed advisable to determine the stability of small amounts of persulfate in flour and dough. It was postulated that amounts of the order of 50 to 200 parts per million might be necessary to mature flour. It follows that an analytical method, to yield useful results, should have quantitative value even at a level as low as 10 parts per million.

The literature reveals no dearth of qualitative color tests for persulfate ion. Aside from the well known benzidine test, various workers have reported the use of fuchsin, brucine, strychnine, iodides, diamino-diphenyl amine, diaminofluorene, aniline sulfate, methylene blue, indigo and ortho-tolidine. Diaminodiphenylamine and diaminofluorene were not available to us. Of the other reagents, only o-tolidine (1) showed up well enough in exploratory tests to merit careful study. This reagent has two important advantages over the commonly used benzidine: not only is it considerably more sensitive, but the blue oxidation product is soluble in water. As little as one microgram of ammonium persulfate in one gram of water oxidizes o-tolidine to a definite blue coloration. However, in one gram of flour, we were unable to detect amounts of persulfate equivalent to less than

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35 or 40 parts per million, and were thus reluctantly forced to abandon this reagent as insufficiently sensitive for our purpose. A number of other potentially useful reagents, not specifically mentioned in the literature, were also investigated. Among these were the leuco (i.e., reduced) forms of malachite green and crystal violet. Neither of these was found to be as sensitive as o-tolidine, but they are mentioned because they suggested the compound eventually chosen.

If colorimetric methods are put aside as being insufficiently sensitive, it becomes natural to search for a fluorometric method. The reason for this is that modern instruments for the quantitative measurement of fluorescence intensities are relatively more sensitive than corresponding instruments for measuring color intensities. Having in mind the behaviour of leuco-crystal violet, it seemed possible that the reduced form of some fluorescent dye might fulfill our requirements. Perhaps the commonest of this class of dyes—the sodium salt of fluorescein—turned out to be eminently satisfactory. Reduced fluorescein is not fluorescent, but is easily re-oxidized to the parent compound which is, of course, very intensely fluorescent.³

Using reduced fluorescein as test reagent, it was found possible to detect as little as one part ammonium persulfate per million of flour. In order to set up reproducible quantitative assay conditions, it was necessary first to investigate methods for separating or extracting minute amounts of persulfate from large amounts of interfering organic substance—i.e., flour. Previous experience with biochemical assay methods suggested the method which will now be described in detail.

Required Reagents

1. Sodium fluorescein. Dissolve 0.2 g. in 100 ml. of water.
2. Sodium potassium tartrate. Dissolve 10 g. of the U.S.P. grade in 100 ml. water.
3. Titanous chloride volumetric solution, 0.04 Normal.

General directions for preparation and storage are given in several texts, of which perhaps only one (3) need be mentioned. The solution must be kept under hydrogen. Analytical grade titanous chloride (20%) distributed by Fisher Scientific Co. has proved very satisfactory as a stock reagent.

4. Zinc sulfate solution, 0.3 Normal.
5. Sodium hydroxide solution, 0.1 Normal.
6. Filtercel (filtering aid).
7. Ammonium persulfate standard solution, 5 micrograms per ml. Dissolve exactly 100 mg. C.P. anhydrous ammonium persulfate in

³ When the work to be described had been practically completed (December, 1948) our attention was drawn to a Russian paper (2) describing leuco-fluorescein as a reagent for oxidizing agents.

exactly 200 ml. distilled water. Dilute 5 ml. of this solution to exactly 500 ml., using distilled water. At the final dilution the reagent does not keep well. It is recommended that it be used within 3 hours of preparation.

8. Reduced fluorescein: given below.

Procedure

I. Setting Up the Standard Curve

To each of five 125 ml. Erlenmeyer flasks add 1.0 g. of untreated (persulfate-free) flour of the type to be tested. Add to each flask 0.5 g. of filtercel and mix. Add, respectively, 34, 33, 32, 30, and 26 ml. of water. Add, respectively, 0, 1, 2, 4, and 8 ml. of the standard ammonium persulfate solution. Add to each flask 4 ml. of the zinc sulfate solution, swirl, and then add 12 ml. of the sodium hydroxide solution. (At this point, the mixture should be very faintly acid. If it is alkaline, the subsequent filtrate will be turbid.) Stopper each flask and shake occasionally during an interval of 15 minutes. Filter each through Reeve Angel No. 230 or equivalent paper. Transfer 10 ml. of clear filtrate from each flask to a fluorimeter tube. Add to each tube exactly 0.2 ml. of reduced fluorescein reagent,⁴ prepared as follows:

Add to a 15 ml. centrifuge tube, in order, 1 ml. stock sodium fluorescein solution, 5 ml. of the sodium potassium tartrate solution, and 4 ml. of the titanous chloride solution. Cap the tube, mix the contents well, and spin in the centrifuge until supernatant is clear (10 minutes at 2,000 r.p.m. seems sufficient).

Mix the contents of each fluorimeter tube, and allow to stand at room temperature for 30 minutes. Set the instrument to zero with the blank flour extract, then read in turn the fluorescence intensity of each extract. The authors use the Coleman Photofluorometer Model 12A with primary filter B-1-S, secondary PC-9. From the readings construct a curve. Figure 1 represents typical curves.

These readings represent the oxidizing effect of extracts of flour treated at levels of 5, 10, 20, and 40 parts ammonium persulfate per million of flour, respectively. The aliquots of filtrate actually used contain only 1, 2, 4, and 8 micrograms ammonium persulfate, respectively. Amounts larger than these produce fluorescence too intense to be measured conveniently.

The flour represented by curve A was vitamin enriched, containing (by actual analysis) in each pound, 2.18 mg. thiamine chloride, 1.36 mg. riboflavin and 17.3 mg. niacin.

⁴ This reagent should be prepared just prior to use. It can just comfortably be made ready during the 15 minute waiting period.

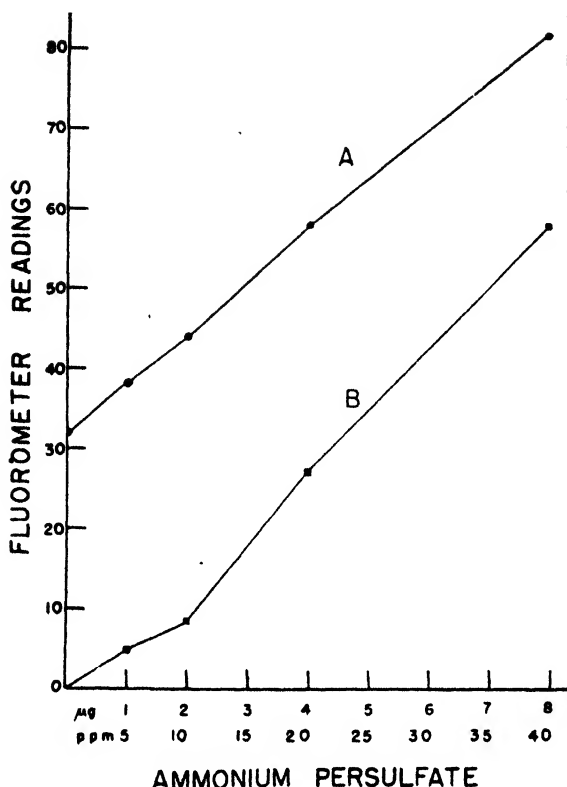


Fig. 1. Determination of ammonium persulfate in flour: fluorometer readings of extracts from standard 1 gram samples. A. Vitamin enriched flour. B. Non-vitamin enriched flour.

Curve B represents a non-enriched flour. The relationship between the two curves will be brought out in the summary discussion.

The intrinsic fluorescence developed by given standard amounts of persulfate varies slightly from day to day, and so it is necessary to run a standard series every day, and preferably, with each series of test samples.

II. Assay of Samples

Along with the standard series already described, process 1-gram samples of the treated flours to be tested, without, of course, adding standard persulfate. If it is known, or suspected, that the treated flours contain more than 40 parts ammonium persulfate per million of flour, then, instead of taking a 10 ml. aliquot of the zinc hydroxide filtrate, take a correspondingly smaller aliquot. In this case, however, make up the difference (i.e., 10 ml. minus aliquot actually taken) with filtrate from the flour blank used in the standard series. This is

important, to compensate for quenching and reduction errors. Plot results from the standard curve.

Portions of dough may be assayed in exactly the same way, with special attention being given to breaking up lumps at the beginning of the test.

Applications of the Test Method

Using the technique described, the stability of ammonium persulfate in non-enriched flour has been determined. Two mixtures, representing different levels of maturing agent, were obtained from the Doty Technical Laboratories, Kansas City, Missouri. These samples, contained in 5 lb. cloth bags, were stored in a laboratory cupboard, with no special precautions, and assayed at intervals. The results are shown in Table I.

TABLE I
THE STABILITY OF AMMONIUM PERSULFATE IN FLOUR

	Sample W-105	Sample W-106
Labeled content, September 24, 1948	66 p.p.m.	132 p.p.m.
Assay of November 3, 1948	70 p.p.m.	135 p.p.m.
Assay of January 24, 1949	70 p.p.m.	140 p.p.m.
Assay of April 12, 1949	68 p.p.m.	145 p.p.m.

The addition of water to flour was found to have a profound effect on the persulfate content. A dough was prepared by adding 60 parts of water to 100 parts of flour which contained 200 parts ammonium persulfate per million. Portions of this mixture were incubated in small closed containers (to conserve water vapor) for various lengths of time at about 27°C. (80°F.) and 37°C. (100°F.) respectively, and then assayed for ammonium persulfate. Table II summarizes the results.

TABLE II
THE STABILITY OF AMMONIUM PERSULFATE IN MOIST FLOUR

Time in minutes	27°C.	37°C.
0	200 p.p.m.	200 p.p.m.
8	55 p.p.m.	17 p.p.m.
15	27 p.p.m.	5 p.p.m.
30	24 p.p.m.	0
45	15 p.p.m.	0
60	0	0

In another test, a dough was prepared by mixing by weight 60 parts water, 1 part sodium propionate, 6 parts dextrose, 2 parts shortening, 2 parts salt, 2 parts yeast, and 100 parts flour which contained 1,000 parts ammonium persulfate per million.⁵ The

⁵ This grossly overdoed flour was used deliberately, to see how much persulfate might survive the test conditions.

sample was incubated at about 27°C. (80°F.) for three hours, at the end of which time an assay showed the residual persulfate to be 7 parts per million. The same sample was then incubated for an additional 30 minutes at about 60°C. (150°F.). At the end of this time an assay showed no persulfate to be present.

Discussion

A method for the assay of ammonium persulfate in flour has been described. The results of a preliminary study indicate that ammonium persulfate is virtually completely stable in normally dry flour, but that it decomposes rapidly in the presence of added water.

A large number of successful assays have convinced the authors that the method has reasonably good precision. Thus, flours carefully prepared so as to contain 100 parts per million ammonium persulfate have been found to assay 100 ± 10 p.p.m. Flours containing only 10 parts per million assay 10 ± 2.5 p.p.m. Readings indicating levels below 5 p.p.m. have little meaning, especially if a control flour of the same type as the unknown is not available. It should be noted that relatively few distinctly different samples of flour were involved in this investigation. It is entirely possible that some types of flour may require modification of the general method. For example, it was noticed that with a very few samples, more than the recommended amount of zinc hydroxide was required to bring about a clear zinc filtrate.

Vitamin enrichment constitutes an obvious source of complication in the method. But, as indicated in curves A and B, Fig. 1, it is easy to make the necessary adjustment. Curve A, representing additions of persulfate to an enriched flour,⁶ is substantially parallel to curve B, representative of a non-enriched flour. Clearly, the presence of vitamin enrichment involves nothing more than a simple additive effect. The "apparent persulfate" value of the normal enrichment concentration (represented by the first point on curve A) amounts to about 23.5 parts per million of flour. Obviously, the simplest way to cancel out the vitamin effect is to use an enriched, non-persulfated flour as the blank when assaying persulfate-matured enriched flours. Even if the enrichment level of this reference sample lies as much as 20% above or below that of the test flour, the net error from this source will probably be not more than ± 5 p.p.m. as persulfate—i.e., 20% of 23.5 p.p.m.

The chemistry involved in the analytical technique is, very briefly, as follows: in the presence of tartrate, fluorescein ($C_{20}H_{12}O_8$) is reduced

⁶ This flour was assayed and found to contain, per pound, 2.18 mg. thiamine chloride, 1.36 mg. riboflavin, and 17.3 mg. niacin.

quantitatively (4) by titanous ion to fluorescein ($C_{20}H_{14}O_6$). The tartrate probably functions both as a buffer and as a reduction catalyst. The fluorescein, which is not fluorescent, is then allowed to react with persulfate ion, and is thereby oxidized back to vividly fluorescing fluorescein. One point, however, is not readily apparent, and should be made clear. A relatively large excess of titanous chloride is used to reduce fluorescein in preparing the reagent. Clearly, if any titanous ion, as such, existed in the reagent at the moment it was added to the flour persulfate extract, a certain amount of the persulfate would react with the trivalent titanium instead of the reduced fluorescein, and thus an apparently low assay would result. As a matter of fact, no titanous ion whatever is left in the reagent after it has been mixed and centrifuged. Presumably, the excess is used up by air in the centrifuge tube. The absence of titanous ion in the prepared reagent was demonstrated by adding 2 ml. of the mixed and centrifuged reagent to 100 ml. of distilled water. The solution was mixed by bubbling purified carbon dioxide (such as used for titanous chloride titrations) through the container for a few seconds. On the addition of one drop of 0.01 *N* methylene blue, the solution became permanently colored.

The effect on the proposed method of concomitant benzoyl peroxide or potassium bromate was studied briefly. Amounts of benzoyl peroxide up to 150 p.p.m. have a negligible effect, if any, on the persulfate assay. Bromate, however, definitely interferes. Three to four p.p.m. of potassium bromate yield an apparent ammonium persulfate content of about one p.p.m.

Acknowledgments

The authors wish to acknowledge their debt to Mr. J. M. Doty for his kindness in preparing and supplying several test mixtures of flour, and to Dr. C. M. Suter of these laboratories for his valuable suggestions, which led to the use of reduced fluorescein as a test reagent.

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A NOTE ON THE OCCURRENCE AND ELIMINATION OF FORMALDEHYDE FUMES FROM A BAKE LABORATORY ATMOSPHERE ¹

D. K. CUNNINGHAM and I. HLYNKA

ABSTRACT

The principal irritant in noxious fumes produced during baking under certain conditions has been identified as formaldehyde formed from the alcohol, vaporized from the loaves during baking, by the catalytic action of open heating elements of nichrome wire in the oven.

Redesigning of our baking laboratory recently involved moving the oven to an ill-ventilated position. Thereafter, noxious fumes irritating to the eyes and respiratory tract became noticeable during baking. A preliminary search of the literature yielded no information on this topic, and an experimental investigation was therefore undertaken.

Preliminary observations on the odor and physiological effect of the fumes indicated that the irritating substance might be formaldehyde. To test this hypothesis the unknown material was concentrated by scrubbing the laboratory air during baking. A suitable scrubbing column consisted of an air filter to remove dust, followed by a sintered glass disc through which air was drawn by suction into a column (20 cm.) of 100 ml. freshly boiled distilled water; unboiled distilled water gave a slight reaction for aldehydes with sensitive reagents.

The unknown solution was tested with Tollens' ammoniacal silver reagent (3) and Schryver's phenyl hydrazine reaction (2). The Tollens' silver reagent was prepared according to an improved method by mixing equal volumes of 10% silver nitrate and sodium hydroxide solutions and then adding ammonium hydroxide dropwise until the brown precipitate just disappeared. When this reagent was added in equal volume to the unknown solution, a brownish color formed and a precipitate of reduced silver was deposited on standing. The test was exceedingly sensitive but required several hours for completion.

The Schryver reagent proved much more adaptable. The original method was modified as follows to improve the sensitivity and reproducibility: To 10 ml. of test liquid were added 2 ml. of 1% phenylhydrazine hydrochloride. This mixture was heated in a boiling water bath for three minutes and cooled to room temperature. One ml. of

¹ Manuscript received August 12, 1949.

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5% potassium ferricyanide was then added. After exactly 30 seconds, 5 ml. of chilled hydrochloric acid were run in and the solution was cooled at once in an ice-bath. A red color formed, which was stable for several days in the cold. This reaction gave a positive indication with as little as 0.02 μ gm. of formaldehyde, and the sensitivity could have been increased by extracting the red substance with water-saturated normal butyl alcohol.

To determine its specificity, the Schryver test was applied to furfural, acetaldehyde, formic acid, and water saturated with carbon dioxide. Furfural gave a brown color, as did acetaldehyde, although the latter faded rapidly; no color reaction was obtained with formic acid. Since large amounts of carbon dioxide are given off in the baking of bread, its effect was tested by passing carbon dioxide generated from dry ice through the apparatus for six hours. No reaction with Schryver's reagent could subsequently be observed in the solution.

The red color developed by both the test solution and formaldehyde on treatment with Schryver's reagent was examined spectrophotometrically. The concentration of formaldehyde used was one μ gm. per ml., which approximated the concentration of unknown in the absorption fluid. Readings were made in a Beckman spectrophotometer at the narrowest slit-width, using 1 cm. corex cells.

Fig. 1 shows the curves obtained by plotting per cent transmission against wavelength over the range 380 to 600 $m\mu$. They are practically identical; the slight variations are due to the difference in concentration of the known and the unknown. The point of minimum transmission for both substances is 523 $m\mu$ and is most clearly defined. A maximum occurs at about 400 $m\mu$ and is attributed to phenylhydrazine-ferricyanide complex.

The demonstrated specificity of Schryver's reagent (1) together with the similarity of the absorption spectra may be regarded as good evidence that the principal irritant in the bake laboratory atmosphere was formaldehyde.

Several hypotheses concerning the source of the formaldehyde were entertained, but it was finally shown that formaldehyde was formed from the alcohol vapors volatilized from yeasted dough during baking. The open heating elements of nichrome resistance wire served as efficient catalysts. Experiments establishing this information are outlined below.

A muffle furnace, lined with an alundum shell and having no open elements or metal parts likely to possess catalytic activity, was set in a closed fume hood to confine the vapors given off during baking. A set of 18 pup loaves was baked in batches of three. Organoleptic judgment by several individuals revealed only the odor of dough and of

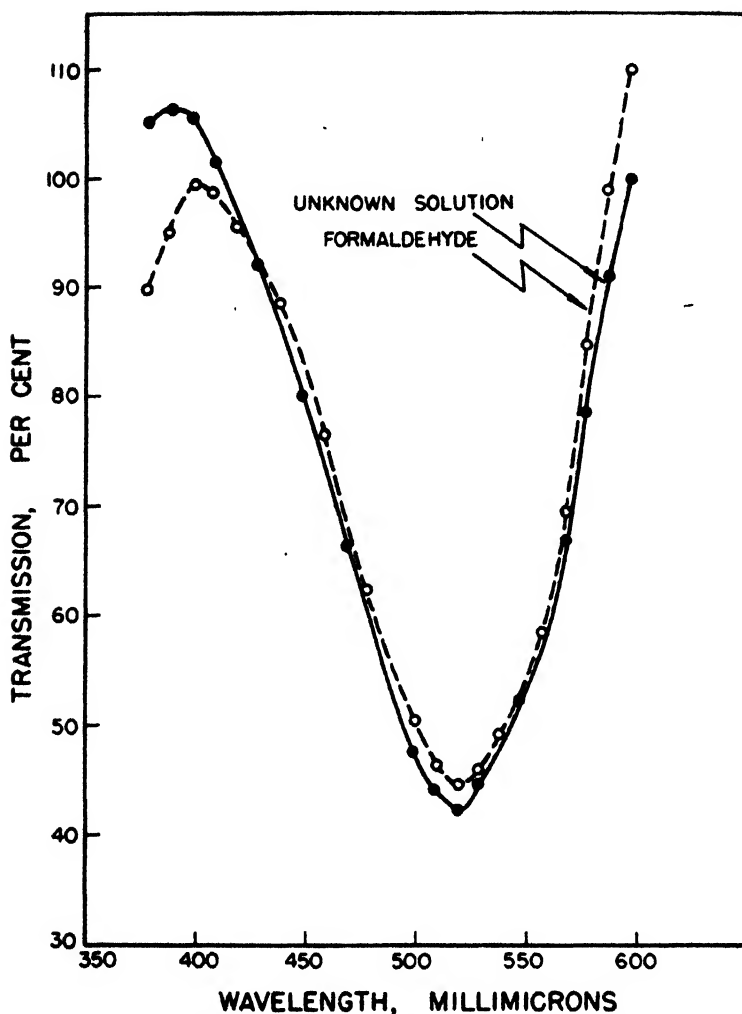


Fig. 1. Comparison of the color complex of formaldehyde and unknown with Schryver's reagent.

freshly baked bread; no irritant was produced. The metal parts of our bake oven were thus definitely implicated.

It was then demonstrated that characteristic acrid fumes were produced by injecting alcohol vapor into the bake oven. A set of 25 doughs, each of approximately 160 g., was prepared by mixing flour with 5% aqueous ethanol. These doughs were panned and fed into the oven, one every five minutes, as is the usual practice. Before long the familiar acrid fumes pervaded the bake laboratory. Ethyl alcohol normally produced in yeast fermentation, was thus the obvious source

of formaldehyde during baking. Confirmatory evidence was obtained incidentally during a recent bread strike in the City of Winnipeg. Several reports describing noxious fumes were obtained from persons who had resorted to home baking. Many of the domestic electric ranges have open heating elements in the oven.

On the basis of the information obtained in this investigation, the open heating elements in the laboratory bake oven were replaced by anodized aluminum-clad strap heaters. Since that time only the pleasant aroma of freshly baked bread pervades the laboratory atmosphere during baking days.

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EFFECT ON DOGS OF FEEDING FLOUR TREATED WITH CHLORINE DIOXIDE AND NITROGEN TRICHLORIDE ¹

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ABSTRACT

Flour treated with chlorine dioxide ranging from 0.61 to 50 g. per hundredweight was fed as a major ingredient in the diet of young and mature dogs for periods ranging from 147 to 355 days.

Young dogs made satisfactory gains in bodyweight while mature dogs were maintained in good physical condition during the tests. Symptoms of canine hysteria were not observed in any of the 12 dogs fed flour treated with chlorine dioxide. All animals remained apparently normal during the tests and showed no clinical abnormalities.

Feeding of flour treated with nitrogen trichloride induced canine hysteria; the time required and the severity of symptoms depended upon the nitrogen trichloride treatment.

It may be summarized that under the condition of experimentation the feeding of flour treated with chlorine dioxide does not induce canine hysteria in either growing or mature dogs.

An affliction, designated by various investigators as canine hysteria, fright fits, and running fits, has been observed under different conditions in dogs during the past 25 years. The exact cause, however, had not been ascertained until Mellanby (3) reported that the feeding of flour treated with nitrogen trichloride (agene), heretofore used widely as an improving agent, developed symptoms of canine hysteria in dogs. The original findings of Mellanby (3) were subsequently confirmed by Mellanby (4), Moran (5), Newell *et al.* (6, 7), Radomski *et al.* (10), and Silver *et al.* (11). The investigation revealed further that rabbits, cats, monkeys, and ferrets are also susceptible to nitrogen trichloride toxicity, while guinea pigs, rats, and chicks were found non-susceptible. No untoward effect has been found when excessive amounts of highly over-treated flour were fed to humans (2, 6, 9).

The fear that the continuous consumption by man of flour treated with nitrogen trichloride may endanger people's health led to exploration for suitable chemical agents free from toxic effects. Among chemical agents studied chlorine dioxide appeared to be the most suitable agent to replace nitrogen trichloride. Newell *et al.* (6) fed flour treated with 2 g. chlorine dioxide per hundredweight to 3 young

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² Raritan Laboratories, Inc., Metuchen, New Jersey.

The authors express their appreciation to the staff members of the laboratory who have helped faithfully to complete the work reported in this paper.

dogs for a period of 84 days without any evidence of toxicity. Radomski *et al.* (10) fed flour treated with 0.6 and 1.8 g. chlorine dioxide per hundredweight to two groups of 3 dogs for a comparatively short period of less than 3 weeks without apparent toxicity. The non-toxicity of flour treated with chlorine dioxide was also observed by Arnold (1) and by Newell *et al.* (8). Arnold fed flour treated with 5 g. chlorine dioxide per hundredweight to 5 young dogs for a period of 16 to 28 days. Newell *et al.*, however, fed flour treated with 0.5 to 4 g. chlorine dioxide per hundredweight to 8 young dogs for a period of 12 weeks. Rabbits, monkeys and rats grew well and appeared normal throughout the tests. Thirteen healthy male and female subjects were fed daily, in addition to a normal diet, 55 g. of wheat proteins treated with chlorine dioxide for a period of 6 months without exhibiting abnormal symptoms.

The object ⁴ of this paper is to present a summary of studies on the feeding of flour treated with chlorine dioxide and nitrogen trichloride. The feeding tests, using dogs, were continued longer than any previously reported studies to determine the effect of the continuous feeding of chlorine dioxide treated flour.

Experimental Method

Young growing and mature dogs were used. The young dogs were immunized against canine distemper during the tests. The mature dogs were previously immunized against distemper. All dogs were clinically normal at the start of the tests. Two dogs were used in each feeding trial.

Stools were checked for parasites once a month, and if positive, rechecked in 10 to 14 days after treatment had been administered. Young dogs were weighed and their temperatures taken twice weekly. Weighing and temperature readings for the mature dogs were done once a week. All dogs were checked daily, in the morning, for general condition such as eye expression, body movement and reflexes, to determine the presence of symptoms of canine hysteria. Samples of blood were examined once a month for hemoglobin concentration, red cell count, white cell count, and Schilling differential counts.

The term "canine hysteria" was used in examination of the test animals to indicate symptoms produced in dogs by feeding flour treated with nitrogen trichloride. Symptoms produced are first general lethargy and muscular incoordination, and later violent running and

⁴ This investigation was supported entirely by Wallace and Tiernan Products, Inc., Newark, New Jersey. The material reported in this publication was taken from the data submitted by Wallace and Tiernan Products at a public hearing held in Washington, D. C. by the Food and Drug Administration in October, 1948. The entire program was carried out in cooperation with Dr. L. Reiner of Wallace and Tiernan Products. The authors wish to express their appreciation to Wallace and Tiernan Products for permission to publish the material reported.

barking fits, and in severe cases clonic convulsions followed by death. In some instances animals in severe condition recover completely as far as general appearance is concerned.

The basal ration had the following ingredients in parts: untreated flour 70, lactalbumin 6, sodium caseinate 6, U.S.P. No. 1 salt mixture 4, dextrose 5, lard 8, sodium carboxymethylcellulose 1, and fish liver oil 0.5 (2000 units A and 400 units D per g.). Each 1000 g. of the basal ration contained the following vitamin supplements: thiamin hydrochloride 21 mg., riboflavin 16 mg., nicotinic acid 105 mg., calcium pantothenate 142 mg., pyridoxine hydrochloride 26 mg., choline chloride 1310 mg., and a trace of Menadione.

The experimental ration was prepared by replacing the untreated flour with the test flour. All rations were prepared at an interval of 10 to 14 days. The dry ration was mixed with boiling water in definite proportions just prior to feeding. Boiling water was added to dextrinize the starch in the wheat flour and to increase palatability. In order to insure an adequate intake of vitamin A, 0.5 cc. fish oil was added to the daily food twice a week. Young growing dogs up to 6 months old were fed two or three times a day. All other dogs were fed once a day. The amount of food fed to each animal was determined by the appetite of the animal. The uneaten portion of food was weighed at the end of the day and the weight of the dry ration was calculated and recorded. In most instances, in order to accustom dogs to the type of rations fed, the basal ration was fed prior to the test. All dogs, however, received a colony ration while not on test. It was formulated and prepared in the laboratory to replace commercial dog food usually fed as a colony ration.

All flours were shipped to the laboratory under code number and their identity was not revealed until all tests were completed.

The description of dogs used and flours fed are summarized in Table I. Flour was treated with 0.61, 0.72, 1.83, and 50 g. chlorine dioxide per hundredweight. Gluten was prepared from flours treated with 0.61 and 1.83 g. chlorine dioxide per hundredweight and was incorporated in the ration at a 15% level, replacing the treated flour. Three rations were prepared with flours treated with 3, 20 and 30 g. nitrogen trichloride per hundredweight.

Results and Discussion

The results of the feeding of rations containing 70% flour treated with either chlorine dioxide or nitrogen trichloride are summarized in Table I. The feeding trials lasted from 147 to 355 days. All animals accepted rations containing chlorine dioxide treated flour. Young

TABLE I
DESCRIPTION OF DOGS AND FLOUR FED
SUMMARY OF FEEDING TRIALS

Dog no.	Description of dog species, sex, age and weight at start of test			Flour in diet	Treatment	No. of days on test	Average daily food offered	Average daily food consumed	Weight change during test	Canine hysteria
				%	per cwt.		g.	g.	kg.	
663	B F ¹	15 mo.	6.00 kg.	70	Untreated	122	209	200	+1.7	None
470	F F ¹	22 mo.	4.50 kg.	70	Untreated	123	199	193	+1.0	None
582	B F	7 mo.	6.5 kg.	70	0.61 g. ClO ₂	189	219	214	+1.0	None
596	F F	5.5 mo.	6.25 kg.	70	0.61 g. ClO ₂	189	207	205	+2.1	None
605	F M	3 mo.	3.5 kg.	55	0.61 g. ClO ₂					
				15	Gluten from same flour	169	174	161	+2.32	None
520	F F	18 mo.	5.5 kg.	55	0.61 g. ClO ₂					
				15	Gluten from same flour	123	229	226	+0.65	None
564	F F	10.5 mo.	4.9 kg.	70	1.83 g. ClO ₂	189	190	184	+0.25	None
594	F F	5 mo.	5.0 kg.	70	1.83 g. ClO ₂	147	186	170	+1.50	None
502	B M	16 mo.	10 kg.	55	1.83 g. ClO ₂					
				15	Gluten from same flour	186	252	248	-0.35	None
538	F M	14 mo.	6 kg.	55	1.83 g. ClO ₂					
				15	Gluten from same flour	186	214	194	+0.05	None
604	F M	4 mo.	5.15 kg.	70	0.715 g. ClO ₂	355	116	103	+4.1	None
327	F F	9 yr.	6.75 kg.	70	0.715 g. ClO ₂	236	206	197	+0.95	None
654	F F	8 mo.	5.35 kg.	70	50 g. ClO ₂	155	210	176	+0.8	None
663	B F	20 mo.	7.9 kg.	70	50 g. ClO ₂	156	181	157	+0.05	None
604	F M	3.4 mo.	5.95 kg.	70	3 g. NCl ₃	21	129	116	+0.5	Present
617	F F	3.4 mo.	1.52 kg.	70	3 g. NCl ₃	21	70	64	+0.23	Present
222	Mongrel F	Mature	6.3 kg.	70	20 g. NCl ₃	13	138	112	+0.1	Present
349	F F	Mature	6.0 kg.	70	Defatted flour treated with 30 g. NCl ₃	10	122	64	-0.8	Present

¹ B indicates Beagle; F, Fox Terrier; M and F, Sex male and female.

dogs made fairly good gains in bodyweight and mature dogs were maintained satisfactorily, regardless of treatment. Two young dogs, 654 and 663, were maintained satisfactorily for a period of 155 days on the ration containing flour treated with 50 g. chlorine dioxide per hundredweight. Fresh horsemeat was added to the daily food of dogs, 596, 604, and 605, in order to encourage good food consumption when these dogs appeared to be suffering from mild bacterial infection.

The total amount of fresh horsemeat fed to each dog was 1.8, 2.45, and 0.3 kg., in order mentioned above. At the end of the tests all dogs, except 594 and 605, were examined by practicing veterinarians and found to be in satisfactory condition from the standpoint of general appearance, quality of coat, and alertness. Dog 594 was found dead on the morning of December 26, 1947. There was no evidence of struggle preceding death. The daily record revealed that for 21 days the dog had a good appetite and her temperature was normal. However, on the 22nd and 23rd day the dog did not consume any food, but there appeared to be no abnormality. Postmortem examination indicated a marked congestion in the lungs, and pneumonia as the probable cause of death. Dog 605 showed symptoms of a low-grade mixed bacterial infection and died of pneumonia after the continuous feeding of the test ration for a period of 174 days.

Symptoms of canine hysteria were not observed in any of the twelve dogs fed flour treated with chlorine dioxide, even when the treatment was increased from 0.61 to 50 g. chlorine dioxide per hundredweight. However, dogs that received rations containing flour treated with nitrogen trichloride developed symptoms of canine hysteria; the number of days required to induce symptoms and the severity of the symptoms depended upon the degree of treatment. The nitrogen trichloride treatment varied from 3 to 30 g. per hundredweight. Dogs 604 and 617 developed hysteria at the end of three weeks' feeding, but recovered. Dog 604 was used subsequently for the feeding of flour treated with chlorine dioxide. Dog 222 developed severe hysteria on the 12th day and was destroyed on the 15th day, due to the severity of the symptoms. Dog 349 developed severe symptoms of hysteria on the 4th day and died the following day. Observations of a similar nature were made in other studies, but the details of these findings are omitted in this report since reports on similar studies appear in the literature.

Monthly blood counts (hemoglobin, red cell and white cell counts and Schilling differential counts) of the dogs remained normal during the tests. There were a few high white cell counts and shifts in the differential counts, but these were associated with bacterial infection, pneumonia, and reaction following the administration of canine distemper vaccine.

Temperatures of the dogs fed rations containing flour treated with various amounts of chlorine dioxide remained within the normal range during the tests. Some elevated temperatures were observed, especially in young growing dogs during the administration of canine distemper vaccine. Elevated temperatures were recorded for dog 605, due to mixed bacterial infection and pneumonia.

At the end of the tests, autopsies were performed on dogs 502, 539, 654, 663, and 604. The animals were sacrificed by bleeding. All organs, brain, liver, spleen, kidneys, lungs, adrenals, thyroids, and pancreas, were removed at once and preserved in standard preservatives. These organs were found to be essentially normal. In some instances slightly enlarged or congested organs were observed, but no gross abnormalities were found in any of the organs examined.⁴

Electroencephalograms were taken at the Bureau of Biological Research, Rutgers University, on dogs, 654, 663, 604, and 327, that were fed with flour treated with chlorine dioxide. Records obtained for the three dogs, 654, 663, and 604, showed no abnormalities. Dog 327, however, showed an abnormal pattern, but it was impossible to determine whether it was due to the diet fed or other unknown factors. Encephalograms were not taken on any of the dogs at the beginning of the tests.

The results reported in this paper and unpublished data from this laboratory confirm the previously published findings that the feeding of flour treated with nitrogen trichloride induces canine hysteria, not only in growing dogs, but also in mature dogs. The severity of symptoms depended upon the degree of treatment, and the number of days on the diet. Symptoms appeared suddenly and animals either died or recovered after a change in the dietary regime, depending upon the severity of the symptoms.

The feeding of flour treated with chlorine dioxide, however, did not induce symptoms of canine hysteria in any of the 12 dogs. Young dogs made fairly good gains in bodyweight and appeared to be in excellent physical condition throughout the tests. Mature dogs were maintained satisfactorily without showing clinical abnormalities, even when flour was treated with 50 g. chlorine dioxide per hundredweight.

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FACTORS AFFECTING THE COLOR OF MACARONI. I. FRACTIONATION OF THE XANTHOPHYLL PIGMENTS OF DURUM WHEATS¹

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ABSTRACT

The carotenoid pigments of semolina made from good and poor varieties of durum wheat, and of macaroni, have been extracted. Partition between 90% methanol and petroleum ether indicates that the pigments are almost entirely hydroxy-carotenoids. Chromatograms on zinc carbonate were similar for all samples and contained two main zones. The most dependable values for the absorption maxima (499 and 470 $m\mu$) of the pigment in the upper zone of the chromatograms, and other supporting evidence, indicate that it is Taraxanthin. Values for the pigment of the lower zone (504 and 473 $m\mu$) indicated that it was not a single compound. Rechromatographing of a larger quantity of lower-zone pigment obtained from a further experiment yielded two zones identified as Isolutein (503.5, 473 $m\mu$) and Xanthophyll (508, 476 $m\mu$). Thus the lower zone of the original chromatograms is considered to be Xanthophyll (also known as Lutein) together with some Isolutein formed during chromatographing. Visual estimation and optical density measurements indicate that there is somewhat more Xanthophyll than Taraxanthin.

Certain varieties of durum wheat, notably Pelissier and Golden Ball, produce macaroni of inferior color. The semolinas made from these varieties contain adequate amounts of yellow pigment but lose a high proportion of it during processing into macaroni. Other varieties, such as Carleton and Mindum, lose less pigment and yield macaroni of better color. As part of an investigation of this difference in behavior, a study was made of the types and amounts of xanthophyll pigments in two semolina samples representing good and poor varieties. A sample of macaroni was also examined to ascertain if any of the pigment fractions were preferentially oxidized during processing.

The true identity of the yellow carotenoid pigments of durum wheats was first established by Markley and Bailey (2). They

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showed that xanthophylls and their esters were the principal components and that carotene was present in much smaller amounts than was originally believed. Munsey (3) later showed that carotene constituted less than 1% of the carotenoid pigments of durum wheat, and he agreed that the principal pigments were xanthophylls. Crystalline Xanthophyll was isolated from Hungarian wheat flour by Zechmeister and Chohnoky (6) who also found little indication of any other important pigment fractions. A successful fractionation of the xanthophylls of durum wheats has not yet been reported in the literature, and it is with this problem that this paper is concerned.

Markley and Bailey (2) and Munsey (3) both attempted to fractionate the xanthophyll pigments extracted from durum wheat but were unsuccessful in obtaining a separation. Since their work was done, considerable improvements in extraction and fractionation techniques have been made by Karrer and his associates (1). Application of these newer methods in this laboratory has now yielded evidence that durum wheat contains both Xanthophyll and Taraxanthin. The procedure involves partition of the carotenoid pigments between petroleum ether and 90% methanol, chromatographing of the xanthophyll fraction in dry benzene on zinc carbonate, and subsequent identification by means of absorption spectra. It would have been preferable to confirm the evidence by isolating the individual pigments as has been done by Zechmeister and Chohnoky (6) with other products. But, as neither time nor equipment were available for the large scale extraction that would be required, it seemed preferable to forego such work in favor of other aspects of the main investigation.

Materials and Methods

When this study was undertaken, essentially as a side issue, only small samples of pure varieties were available for study. Absorption spectra of extracts of samples of Mindum and Stewart showed identical maxima, and as these varieties are of similar parentage it was considered legitimate to combine them to obtain a larger representative sample of good macaroni-making quality. Pelissier was available as a representative variety of poor quality. Semolina was made from both samples by the usual laboratory mill procedure. A reasonably large sample of macaroni made from pure Mindum was also available. It was ground to pass a 60 mesh sieve to yield a sample of similar granulation to the semolinas.

The method outlined by Karrer *et al.* (1) was followed in detail. The adsorption column used for the chromatographic analysis was 8 inches long and $\frac{3}{4}$ inch in diameter. The extent of development was the only variable among experiments with the three samples investi-

gated. This was varied in accordance with the amount of pigment obtained for chromatographing and also, to some extent, as additional experience was gained with the technique. The size of the samples extracted were: good variety, 500 g.; poor variety, 600 g.; and macaroni, 1,000 g. The samples were examined in this order, and the development of the chromatograms increased with each successive sample. The pigments in the bands were eluted with methanol and absorption spectra were determined in carbon disulfide with a Beckman Spectrophotometer.

In order to obtain enough material for rechromatographing of the principal pigment zone, a further study was made with 1,500 g. of semolina milled from a sample of 3 C.W. Amber Durum containing a mixture of varieties. This experiment is discussed separately.

Results and Discussion

The method involves extraction of the original material with methanol and with petroleum ether. After suitable treatment, the pigments in these two extracts are partitioned between 90% methanol and petroleum ether so as to separate the xanthophylls from the other carotenoids. With each of the three samples, all but a trace of the pigments were found in the methanol layers. That is, the great bulk of the pigments are xanthophylls. These results are in agreement with those of Markley and Bailey, and of Munsey.

The three chromatograms were almost identical in appearance. Each exhibited four bands. The uppermost band was only about one millimeter in depth, and was brownish in color. In all cases it was very tenaciously adsorbed and did not move down the column during development of the chromatogram. There was a clear region between this strongly adsorbed layer and the next colored band (zone 2). The second band was reasonably well defined, about five millimeters in depth, and yellow-orange in color. This band was followed by a region of a few centimeters depth which had a very pale yellowish color. This was not a region of strong adsorption, but appeared to contain mostly pigment washed down from zone 2. The final band (zone 4) was also reasonably well defined, slightly deeper than zone 2, and orange in color. Zones 2 and 4 contained the bulk of the pigments.

No quantitative determinations were made of the relative concentrations of the pigments of zones 2 and 4 of these chromatograms, but from a visual assessment of the relative depth and intensity of these bands, it appeared that the lower band (zone 4) contained slightly more pigment than the upper band (zone 2). This ratio appeared to be the same for all three chromatograms.

The amounts of pigments in the individual zones were too small

to permit further purification. This would involve rechromatographing, crystallization of the pigment, and final identification by melting point, optical rotation, and absorption maxima. In the present study only the absorption maxima could be determined. These are shown in Table I. The following discussion deals almost wholly with zones 2 and 4 which contained the bulk of the pigments. Three questions arise: Are the pigments obtained in each of the three experiments identical? If so, which experiment yields the most accurate data? And do these data agree with recorded maxima for known pigments?

TABLE I
ABSORPTION MAXIMA ($m\mu$) FOR PIGMENTS ELUTED
FROM EACH CHROMATOGRAM ZONE

Zone ¹	Semolina		Macaroni Mindum (Exp. 3)
	Mindum-Stewart (Exp. 1)	Pelissier (Exp. 2)	
1	498	415	381
	469	383	
2	501	498.5	499
	471.5	469.5	
3	500	500	500
	470	470	
4	504	503.5	503
	473.5	473	

¹ The zones are numbered from the top of the column.

Since the Beckman instrument yields data that are accurate within less than $\pm 0.5 m\mu$, the principal consideration is the purity of the pigment in each zone. This depends primarily on the efficiency of development of the three chromatograms, which was poorest for experiment 1 and best for experiment 3. Accordingly, it is believed that the pigment in zone 2 was obtained in purest form from experiment 3. The uppermost zone, which was invariably adsorbed tenaciously, was washed free of all pigments adsorbed lower, and zone 2 was also washed free of pigments adsorbed in zone 4. Almost as much development probably occurred in experiment 2 for which the zone 2 data are similar to those of experiment 3. But in experiment 1, it appears that zone 2 was not washed free of zone 4 pigments and therefore yields higher results. The opposite situation holds for the pigment of zone 4; it was least contaminated with pigments from zone 2 in the least developed chromatogram of experiment 1, and most contaminated in experiment 3. All data for zones 2 and 4 thus appear to be consistent with the hypothesis that all three samples contained

the same two principal pigments, one of which is adsorbed in zone 2 and the other in zone 4. The most reliable data for the absorption maxima are considered to be:

Pigment of zone 2 — 499 $m\mu$ and 470 $m\mu$;

Pigment of zone 4 — 504 $m\mu$ and 473.5 $m\mu$.

The pigment of zone 1 was very strongly adsorbed as was demonstrated by its failure to move down the tube at all during development. Accordingly, it is improbable that zone 2 was contaminated by any pigment from zone 1. But zones 2 and 4, and zone 3 which is thought to have been a mixture of pigments from zones 2 and 4, were less strongly adsorbed. Thus it seems probable that zone 4 was contaminated with some zone 2 pigment even in the least developed and certainly in the most developed chromatogram. Accordingly, though the maxima given above for zone 2 are probably reasonably accurate, those for zone 4 may be shifted towards the ultraviolet by contamination.

The known hydroxy-carotenoid pigments with absorption maxima similar to those of zone 2 pigments are:

Taraxanthin — 499 $m\mu$ and 470 $m\mu$;

Violaxanthin — 500.5 $m\mu$ and 469 $m\mu$;

Xanthophyll-monoepoxide — 501 $m\mu$ and 472 $m\mu$.

Maxima for the zone 2 pigment agree best with those for Taraxanthin. Additional evidence that the unknown is neither of the other two compounds was obtained by color tests. In ether solution, Violaxanthin gives a blue color with hydrochloric acid, whereas Taraxanthin does not. When treated with chloroform that has stood long enough to develop a slight amount of hydrochloric acid impurity, Xanthophyll-monoepoxide undergoes a molecular rearrangement with a shift in the absorption maxima to 479 $m\mu$ and 449 $m\mu$ (Karrer, 1). The zone 2 pigments (and those from all other zones) gave negative results for both tests. The pigment of zone 2 is therefore considered to be Taraxanthin, $C_{40}H_{56}O_4$.

Identification of zone 4 presents greater difficulty. Its maxima, 504 and 473.5 $m\mu$, are close to those reported by earlier workers for Leaf Xanthophyll, 505 and 473 $m\mu$. About 15 years ago Leaf Xanthophyll (or simply Xanthophyll) was thought to be one of two isomers; the other was called Lutein. The absorption maxima for these two pigments were given (5) as 505 and 473, and 508 and 475 $m\mu$. Strain (4) has since suggested that Leaf Xanthophyll is a mixture, whereas Lutein is a single compound. The situation is further complicated because the pigment having maxima at 508 and 475 $m\mu$ is now called Xanthophyll by Karrer and his co-workers, whereas the name Lutein

is retained elsewhere. Since Karrer's methods were used in the present investigation, his nomenclature has been adopted.

The literature also shows that Xanthophyll is often difficult to separate, either by recrystallization or rechromatographing, from other similar pigments. Moreover, Strain (4) finds that on chromatographing *pure* Xanthophyll small amounts of a new pigment are formed. He calls this Isolutein and reports that it is adsorbed above the Xanthophyll and has maxima at 503 and 473 $m\mu$.

These considerations suggested that rechromatographing of zone 4 might aid in its identification. A larger sample of semolina, milled from 3 C.W. Amber Durum wheat containing a mixture of varieties, was therefore treated by the method previously described. The initial chromatogram did not develop as effectively as had the other three, although a sufficient separation of zone 4 was effected. Rechromatographing of zone 4 on a smaller column yielded only one zone with maxima at 505 and 473 $m\mu$. It was again removed and rechromatographed once more. This time, after prolonged development, two bands were obtained. The lower band exhibited maxima at 508 and 476 $m\mu$ which agree almost exactly with those for Xanthophyll, 508 and 475 $m\mu$. The upper band had maxima at 503.5 and 473 $m\mu$ which again agree almost exactly with those for Isolutein, 503 and 473 $m\mu$. Accordingly, zone 4 of all chromatograms is thought to consist mainly of Xanthophyll together with Isolutein formed on the column during the development of the chromatograms.

The relative amounts of pigment in the two main zones (2 and 4) of the initial chromatogram for the large sample of semolina were estimated from the optical densities of the pigment solutions. The ratio was one part of Taraxanthin (zone 2) to 1.35 parts of Xanthophyll (zone 4). This estimate must be considered approximate, but is also supported by visual inspection which suggested that there was slightly more Xanthophyll (zone 4) than Taraxanthin (zone 2) in each of the three original chromatograms.

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REACTION OF DOUGH AND GLUTEN WITH GLUCOSE¹

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ABSTRACT

The application of the Chapman and McFarlane ferrometric reduction test to studies on dough and gluten shows that a reaction with glucose takes place. The following are typical experiments on materials stored for 5 months at laboratory temperature: Flour and flour mixed dry with 5% glucose had reducing values of 3.7×10^{-6} and 3.3×10^{-6} expressed as moles of ferrocyanide per gram sample, dry weight basis. Flour with 5% glucose, made into a dough, then air-dried and ground, showed a reducing value of 1.5×10^{-6} or a fourfold increase. Analogous experiments with gluten showed an eightfold increase in reducing value. It was also established that a storage period was necessary to bring about the glucose-protein interaction. Samples of flour with glucose, made into a dough, air-dried, and ground, showed no increase in reducing value over undoughed samples when assayed within several days. It was also shown that the reducing value of gluten was essentially unchanged by washing the gluten to remove the added and reacted glucose.

This evidence supports the hypothesis that reducing carbohydrates in dough and gluten may act as cross-linking agents between protein chains to form a three-dimensional elastomer network.

Observations on the behavior of dough and gluten towards such reagents as bisulfite and acetaldehyde led the writer (6) to suggest that a chemical interaction between wheat proteins and reducing carbohydrates was likely. To test this hypothesis, a method of measuring the carbohydrate-protein complex was necessary. One such method became available when Lea (9) established that the sugar-protein complex possessed reducing activity in the Chapman and McFarlane (2) ferrometric reduction test. This communication describes the application of the Chapman and McFarlane test to a study of dough and gluten.

Interest in the sugar-protein reaction, and a series of reactions initiated by it, has been wide and varied. In 1912 Maillard (10) noted that reducing sugars and amino acids reacted to yield a colored product which he termed melanoidin. Kostychew and Brilliant (8) observed a diminution in amino nitrogen on reacting protein hydrolyzates or amino acids with glucose. Further, the work of Frankel and Katchalsky (5) showed that this reaction was slow and could be

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determined titrimetrically. Bate-Smith and Hawthorne (1) associated the loss of glucose, and Olcott and Dutton (13) the appearance of brown discoloration, in dried egg products, with the condensation of glucose and protein. Also working with dried egg products, Pearce, Thistle, and Reid (14) developed a method of measuring the fluorescing substances which are formed. A similar interaction of cheese proteins with glucose was described by Hlynka and Hood (7) who produced a brown discoloration and observed changes in acidity in process cheese to which glucose was added. Finally, an important contribution to this subject has been made by Olcott and his co-workers (4, 11, 12). They established that aldehydes can act as cross-linking agents between functional groups in proteins and indicated that the cross-linking reaction is associated with the so-called browning reaction. It is now generally recognized that the complex series of reactions, initiated by the sugar-protein interaction, is of common occurrence especially where prolonged storage or heat processing is involved. Preliminary evidence is presented for the occurrence of this reaction between glucose, on the one hand, and flour or gluten, on the other.

Materials and Methods

Straight grade flour was experimentally milled from composite samples of Rescue and Thatcher wheat from the 1946-47 crop. Gluten was washed out by hand from Thatcher flour, air-dried, and ground to go through a No. 40 Wiley sieve. The following materials were prepared:

Untreated: Rescue flour, Thatcher flour, Thatcher gluten.

Dry mixtures of flour or gluten with glucose: Thatcher flour + 2% glucose, Thatcher flour + 5% glucose, Thatcher gluten + 2% glucose, Thatcher gluten + 5% glucose.

Mixtures with glucose of flour made into dough and gluten reconstituted with water, air-dried, and ground: Thatcher flour + 2% glucose, Thatcher flour + 5% glucose, Thatcher gluten + 2% glucose, Thatcher gluten + 5% glucose.

At the time of preparation of samples, the flour was about 6 months old. All the samples were then stored for about 5 months in the laboratory and reducing values were determined at the end of storage period.

Material was thus available for the determination of reducing values of flour and gluten, and of two types of flour and gluten mixtures with glucose. The first type contained dry glucose mixed with gluten or flour so that the effect of the presence of glucose on reducing value could be ascertained. In the second type of mixture, glucose with flour or gluten were wetted to make dough or reconstituted gluten,

and moisture was reduced to approximately that of the original products by air drying. These samples would thus allow reaction between glucose and flour or gluten under conditions similar to those in stored flour.

The method of determining reducing values is essentially that of Chapman and McFarlane, with several modifications. Since flour and gluten do not dissolve in the reagents used, but form a heterogeneous system, continuous mechanical stirring is necessary. A 1-minute period was adopted for the development of color with ferric chloride in the test sample. Lastly, since there is some turbidity in the final solution in which the color is measured, a blank identical with the test solution, but with an equivalent amount of water replacing the ferric chloride solution, was adopted to eliminate the influence of turbidity on colorimeter readings.

The procedure was as follows: To a suitable sample of flour or gluten in a 50-ml. centrifuge tube are added 5 ml. distilled water at 70°C. and the sample is stirred briskly into suspension. Then 5 ml. of potassium acid phthalate-sodium hydroxide buffer, pH 5, and 5 ml. of a 1% solution of potassium ferricyanide are added. Sometimes gluten will form lumps instead of a uniform suspension, but this difficulty is overcome if the water is added last. The centrifuge tube is then placed in a 70°C. water bath, and the same stirring rod that was used for agitation is now secured into the chuck of a stirring motor. The reaction mixture is heated at 70°C. for exactly 20 minutes with continuous power agitation, then transferred to an ice bath and allowed to cool to 25°C. Five ml. of 10% trichloroacetic acid are added, the sample is centrifuged for 1 minute and filtered through a No. 1 Whatman filter paper. Five ml. of the solution are transferred to each of two colorimeter tubes. To one of these tubes are added 6 ml. distilled water and this tube is used as a blank with which the Evelyn colorimeter is set to read 100. To the second tube are added 5 ml. distilled water and 1 ml. freshly prepared 0.1% ferric chloride solution. One minute is allowed for the color to develop before reading is made. Colorimeter readings, made with a 660 m μ filter, are converted to reducing values in terms of moles of ferrocyanide by means of a calibration chart previously prepared. The final results are all reduced to a one gram, dry weight basis.

Results and Discussion

The experiments described may be divided into two parts, those with flour for which the data are summarized in Table I, and those with gluten for which the data are in Table II. The results are ex-

TABLE I
REDUCING VALUES OF FLOUR, VARIOUSLY TREATED

Expt. No.	Material	Treatment	Reducing value moles $K_4Fe(CN)_6/g.$ $\times 10^{-5}$
1	Rescue flour	None	0.32
2	Thatcher flour	None	0.37
3	No. 2	At end of storage, wetted, air-dried, analyzed within 24 hours	0.36
4	No. 2 plus 2% glucose	Mixed dry, stored	0.37
5	No. 2 plus 2% glucose	Mixed, wetted, re-dried, stored	0.95
6	No. 2 plus 5% glucose	Mixed dry, stored	0.33
7	No. 2 plus 5% glucose	Mixed, wetted, re-dried, stored	1.50
8	No. 6	At end of storage, wetted, air-dried, analyzed within 24 hours	0.36

pressed as moles of ferrocyanide formed by the reducing action of the sample in the Chapman and McFarlane test.

An examination of the data in Table I shows that the reducing values for Rescue and Thatcher flour are nearly the same. Nor did 6 months storage or wetting and air drying (Experiment 3) have much effect. Experiments 4 and 6 establish that glucose does not act as a reducing substance in the Chapman and McFarlane test. However, when intimate contact was provided between flour and glucose by wetting, followed by air drying and storing the samples, as in Experiments 5 and 7, reducing values were greatly increased. The sample containing 2% glucose showed a 2.5-fold increase, and the 5% glucose sample a 4-fold increase. Finally, experiment 8 again shows that mere wetting of glucose and flour mixture and air drying it produces no marked increase in reducing value.

The results on reducing values in gluten experiments, summarized in Table II, are very similar to those of flour experiments. It will be

TABLE II
REDUCING VALUES OF GLUTEN, VARIOUSLY TREATED

Expt. No.	Material	Treatment	Reducing value moles $K_4Fe(CN)_6/g.$ $\times 10^{-5}$
9	Thatcher gluten	Air-dried, stored	1.2
10	No. 9	At end of storage, wetted, air-dried, analyzed within 24 hours	1.2
11	No. 9 plus 2% glucose	Mixed dry, stored	1.1
12	No. 9 plus 2% glucose	Mixed, wetted, re-dried, and stored	6.5
13	No. 9 plus 5% glucose	Mixed dry, stored	1.1
14	No. 9 plus 5% glucose	Mixed, wetted, re-dried, and stored	9.8
15	No. 13	At end of storage, wetted, re-dried, analyzed within 24 hours	1.1

noted that gluten alone has a reducing value of about three times that of flour. Experiments 11 and 13 again show that glucose is not a reducing agent in the test, and Nos. 10 and 15 show that wetting and redrying of gluten also fail to enhance reducing values. Gluten, however, showed a higher reactivity with glucose than did flour. The 2% glucose sample (No. 12) showed a 4-fold, and the 5% glucose sample (No. 14) an 8-fold increase in reducing value compared with the original gluten. The reducing activity of these samples is apparently associated with the gluten, since washing gluten-glucose samples under the tap did not markedly decrease reducing values.

The inferences which may be drawn from the data are as follows. If Lea's conclusion, since confirmed by Crowe, Jenness, and Coulter (3), that the Chapman and McFarlane test measures sugar-protein complex formation is accepted, it follows that this type of reaction occurs between glucose and flour proteins. There is also the less likely alternative that it is the gluten lipids that react rather than the proteins. No specific data are available on this point.

Since small amounts of reducing carbohydrates are naturally present in flour it is logical that these should react with flour proteins in the same way as added glucose. Such interaction would be expected, especially in flour that has been stored for some time. Also judging from the high optimum water content which has been reported for this type of reaction, the reducing carbohydrate-protein interaction should be more pronounced in dough than in flour. However, doughs are not usually kept for long periods and the extent of the reaction might not be large. Nevertheless, the reducing property of the carbohydrate-protein complex suggests that it may be a hitherto unrecognized source of reducing activity in flour.

In summary, the results obtained in this investigation further support the hypothesis (6) that reducing groups in carbohydrates may act as cross-linking agents between protein chains in the elaboration of a three-dimensional network in dough and gluten. Such a hypothesis would thus relate the carbohydrate-protein interaction with the physical properties of dough and gluten in terms of their ultimate structure. Further studies on the reaction of glucose with gluten and dough are in progress to obtain further information on the questions raised in this investigation.

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